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Thème

Kinetic Investigation of Protein Adsorption on Porous Surfaces

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Electrons will not change their orbit without energy, and success
will not be written without hardship.

A long journey in the world of physics every transition within it
required force, and every stability within it was preceded by
turbulence until an ending was written, in whose writing everyone
who supported

me took part, and who were my rain after the drought.

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To my parents you were the warm embrace that held me and my
sister I dedicate this achievement to you.

Abstract

This work aims to investigate the adsorption of bovine serum albumin (BSA) on different surfaces, namely silicon, germanium, and porous titanium dioxide (TiO₂), in order to understand the mechanisms governing protein–surface interactions and to evaluate the effect of porous structures on adsorption behavior.

In the theoretical part, the fundamental concepts related to proteins, porous surfaces, and the adsorption phenomenon were reviewed, with particular emphasis on titanium dioxide because of its unique physical, chemical, and biological properties. Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode (FTIR-ATR) was also presented as an effective technique for monitoring the adsorption process and analyzing the structural changes occurring in proteins during their interaction with solid surfaces. In addition, classical kinetic models commonly used to describe adsorption kinetics were examined, including the Pseudo-First-Order (PFO), Pseudo-Second-Order (PSO), and Intraparticle Diffusion models.

In the experimental part, porous titanium dioxide thin films were prepared on germanium substrates using the drop-casting technique. Adsorption experiments of BSA were then carried out on silicon, germanium, and porous titanium dioxide surfaces. The obtained results were analyzed using both classical kinetic models and a proposed Three-State Kinetic Model developed to describe the complex and multistep nature of protein adsorption.

The results showed that the adsorption process is strongly influenced by the nature of the surface and that classical kinetic models are not sufficient to accurately describe all stages of the process. In contrast, the Three-State Model provided the best agreement with the experimental data for all investigated surfaces, confirming that protein adsorption proceeds through several successive stages involving molecular transport in the bulk solution, surface adsorption, molecular rearrangement, and diffusion into the pores.

The study also demonstrated that porous titanium dioxide exhibits a higher adsorption capacity than non-porous surfaces such as silicon and germanium. This behavior is attributed to the contribution of the internal pores, which provide additional sites for protein retention and facilitate diffusion within the porous structure. Overall, the proposed model offers a more accurate and realistic description of protein adsorption kinetics and represents an important step toward a better understanding of biological interactions at solid surfaces, thereby contributing to the development of more effective medical and technological applications.

Key words: protein adsorption, porous surface, TiO₂, BSA, Three state model, Kinetics models

Résumé

Ce travail vise à étudier l'adsorption de l'albumine sérique bovine (BSA) sur différentes surfaces, notamment le silicium, le germanium et le dioxyde de titane poreux (TiO_2), afin de comprendre les mécanismes d'interaction de la protéine avec ces surfaces et d'étudier l'influence de la structure poreuse sur le comportement d'adsorption. Sur le plan théorique, l'étude aborde des concepts fondamentaux liés aux protéines, aux surfaces poreuses et à l'adsorption, en se concentrant sur le dioxyde de titane en raison de ses propriétés physiques, chimiques et biologiques uniques. La spectroscopie infrarouge à transformée de Fourier (FTIR-ATR) est présentée comme une technique efficace pour suivre le processus d'adsorption et analyser les modifications structurales de la protéine lors de son interaction avec la surface. De plus, les modèles cinétiques classiques utilisés pour décrire la cinétique d'adsorption sont examinés, tels que le modèle de pseudo-premier ordre (PFO), le modèle de pseudo-second ordre (PSO), et le modèle de diffusion intrapore. Sur le plan pratique, des films minces de dioxyde de titane poreux ont été préparés sur des substrats de germanium par dépôt par goutte. Par la suite, des expériences d'adsorption de la protéine BSA ont été menées sur des surfaces de silicium, de germanium et de dioxyde de titane poreux. Les résultats ont été analysés à l'aide de modèles cinétiques classiques ainsi que d'un modèle cinétique à trois états, développé pour décrire la nature complexe et multi-étapes de l'adsorption des protéines. Les résultats ont montré que le processus d'adsorption est fortement influencé par les propriétés de surface et que les modèles classiques sont inadaptés pour décrire précisément toutes les étapes du processus. En revanche, le modèle à trois états a présenté la meilleure concordance avec les résultats expérimentaux pour toutes les surfaces étudiées, confirmant que l'adsorption des protéines se déroule en plusieurs étapes successives : adsorption en solution, adsorption de surface, réarrangement moléculaire et diffusion au sein des pores. L'étude a également démontré que le dioxyde de titane poreux possède une capacité d'adsorption supérieure à celle des surfaces non poreuses telles que le silicium et le germanium, grâce à la contribution des pores internes à la rétention et à la diffusion des protéines au sein de la structure poreuse. De manière générale, le modèle proposé offre une description plus précise et réaliste de la cinétique des protéines et représente une avancée importante vers la compréhension des interactions biologiques sur les surfaces solides et le développement d'applications plus efficaces dans les domaines médical et technologique.

Mots clés : adsorption de protéines, surface poreuse, TiO_2 , BSA, modèle à trois états, modèles cinétiques

المخلص

يهدف هذا العمل إلى دراسة امتزاز بروتين الألبومين المصلي البقري (BSA) على أسطح مختلفة تتمثل في السيليكون والجرمانيوم وثاني أكسيد التيتانيوم المسامي (TiO_2) ، وذلك من أجل فهم آليات تفاعل البروتين مع الأسطح المختلفة ودراسة تأثير البنية المسامية على سلوك الانتصااص.

في الجانب النظري، تم التطرق إلى المفاهيم الأساسية المتعلقة بالبروتينات والأسطح المسامية وظاهرة الانتصااص، مع التركيز على ثاني أكسيد التيتانيوم نظراً لخصائصه الفيزيائية والكيميائية والحيوية المميزة. كما تم عرض تقنية مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR-ATR) باعتبارها تقنية فعالة لمتابعة عملية الامتزاز وتحليل التغيرات البنيوية التي تطرأ على البروتين أثناء التفاعل مع السطح. بالإضافة إلى ذلك، تمت دراسة النماذج الحركية الكلاسيكية المستعملة لوصف حركية الامتزاز، مثل نموذج الرتبة الأولى الزائفة (PFO) ، ونموذج الرتبة الثانية الزائفة (PSO) ، ونموذج الانتشار داخل المسام.

أما في الجانب التطبيقي، فقد تم تحضير أغشية رقيقة مسامية من ثاني أكسيد التيتانيوم على ركائز من الجرمانيوم باستعمال تقنية الإسقاط بالتقطير (Drop-casting). بعد ذلك أُجريت تجارب امتزاز بروتين BSA على أسطح السيليكون والجرمانيوم وثاني أكسيد التيتانيوم المسامي، وتم تحليل النتائج باستعمال النماذج الحركية الكلاسيكية بالإضافة إلى نموذج حركي مقترح ثلاثي الحالات تم تطويره لوصف الطبيعة المعقدة ومتعددة المراحل لعملية الامتزاز البروتيني.

أظهرت النتائج أن عملية الامتزاز تتأثر بشكل كبير بطبيعة السطح، وأن النماذج الكلاسيكية غير كافية لوصف جميع مراحل العملية بدقة. في المقابل، أظهر نموذج الحالات الثلاث أفضل توافق مع النتائج التجريبية بالنسبة لجميع الأسطح المدروسة، مما يؤكد أن انتصااص البروتين يمر بعدة مراحل متتالية تشمل انتقال الجزيئات داخل المحلول، ثم الامتزاز السطحي، يليه إعادة الترتيب الجزيئي والانتشار داخل المسام.

كما بينت الدراسة أن ثاني أكسيد التيتانيوم المسامي يمتلك قدرة امتزاز أعلى مقارنة بالأسطح غير المسامية مثل السيليكون والجرمانيوم، وذلك بسبب مساهمة المسام الداخلية في احتجاز البروتينات وانتشارها داخل البنية المسامية. وبصفة عامة، يوفر النموذج المقترح وصفاً أكثر دقة وواقعية لحركية انتصااص البروتينات، ويمثل خطوة مهمة لفهم التفاعلات الحيوية على الأسطح الصلبة وتطوير تطبيقات أكثر فعالية في المجالات الطبية والتكنولوجية.

الكلمات المفتاحية: امتصااص البروتينات، السطح المسامي، ثاني أكسيد التيتانيوم، بروتين الألبوم البشري (BSA)، نموذج ثلاثي الحالات، النماذج الحركية

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General Introduction

Protein–surface interaction is considered one of the most important topics due to its fundamental role in many medical and technological applications , such as medical implants and artificial tissue [1,2]. Porous surfaces are among the most widely used materials in this field, including titanium dioxide (TiO₂), which has received extensive attention due to its distinctive physical and biological properties, such as porous structure, chemical stability, and biocompatibility [3]. Silicon and germanium surfaces are also used in many spectral and electronic applications due to their important optical and electronic properties [4].

Bovine serum albumin (BSA) protein is widely used as a common model in adsorption studies, due to its structural stability, ease of availability, and low cost, in addition to the fact that its physical and chemical properties are well known [5]. The study of the adsorption of this protein on porous and non-porous surfaces is an important step to understand adsorption mechanisms and the behavior of proteins during this complex process.

Protein adsorption is affected by several driving forces , including the nature of the surface, the porous structure, electrostatic interactions, in addition to the movement of molecules inside the pores [1]. To study this phenomenon, advanced analytical techniques are used such as Fourier Transform Infrared Spectroscopy (FTIR), which allows monitoring the adsorption process and the structural changes that occur in the protein during its interaction with the surface [6].

To describe the adsorption behavior, classical kinetic models them (pseudo-first order model, pseudo-second order model, Elovich model and intraparticle diffusion model) are used; however, these models are limited in explaining the complex mechanisms of proteins, as they do not show all stages of adsorption and molecular transport inside the pores. This is what has led to the need to propose the three-state model as it describes adsorption behavior more accurately and realistically.

This work aims to study the adsorption of BSA protein on different surfaces including silicon, germanium, and porous titanium dioxide, with analysis of the kinetic behavior of the adsorption process and understanding the effect of the porous structure on the interaction mechanism.

This manuscript is divided into two main chapters; the first one deals with a theoretical study of protein adsorption on porous surfaces, proteins, adsorption mechanisms, FTIR technique, in addition to the kinetic models used in the study of adsorption. The second chapter includes the experimental part, preparation of thin films of titanium dioxide, as well

General Introduction

as presenting the results and discussing them using different kinetic models especially the proposed one.

CHAPTER 1

Surface – Protein interactions

1 Introduction

Protein adsorption is a complex process of great importance, as it plays a significant role in various biological and technological applications [1], including biomaterials, biosensors, implants [7], cardiovascular systems and drug delivery systems [8]. Titanium dioxide (TiO_2) is considered one of the most important materials for studying protein–surface interactions due to its favorable physicochemical properties [3]. Bovine serum albumin (BSA) is used as a model protein due to its stability, availability, and well-known structure [5]. To better understand this complex phenomenon, analytical techniques such as Fourier Transform Infrared (FTIR) spectroscopy are employed to study protein adsorption kinetics. In addition, kinetic models are used to describe the mechanisms governing this process.

2 Porous surfaces

A porous surface is a material that contains voids, where porosity increases the surface area available for interaction with external substances [9]. There are three categories of porous materials according to IUPAC (International Union of Pure and Applied Chemistry), based on pore size, Macroporous materials contain large pores (up to 500 Å) and have a lower surface area compared to other types. An example of them is porous glass, and they are used in filtration processes and as catalyst supports. Microporous materials have pores smaller than 20 Å and are suitable only for small molecules. An example of them is zeolites such as faujasite, which are used in catalytic processes and gas separation. As for mesoporous materials, they have pores whose size falls within the range between 20 and 500 Å. They are characterized by a high surface area and a uniform pore distribution, such as (SBA-15), in addition to the possibility of controlling pore size. This makes them ideal for the adsorption of proteins such as bovine serum albumin (BSA), and they have applications in drug delivery, catalysis, and biomedical materials [10]. Porous surfaces can also be classified in two ways: natural materials such as clay and zeolite, and synthetic materials such as activated carbon [11]. Among these porous surfaces, titanium dioxide (TiO_2) was selected as a model for studying protein adsorption. Porous surfaces can also be classified in two ways : natural materials such as clay and zeolite, and synthetic materials such as activated carbon [11]. Among these porous surfaces, titanium dioxide was selected as a model for studying protein adsorption.

2.1 Titanium dioxide (TiO₂)

Titanium dioxide has a highly porous structure and a spherical shape with a high specific surface area and an ordered structure and biocompatibility, it has attracted great interest because it is suitable for many applications such as catalysis, sensing, and biological applications [3,12]. It is considered one of the most suitable semiconductors, it has a band gap energy of about 3 electron volts [13]. Titanium dioxide has three phases: rutile, brookite, and anatase. Anatase is considered the most stable and is easier to synthesize compared to the other phases [14].

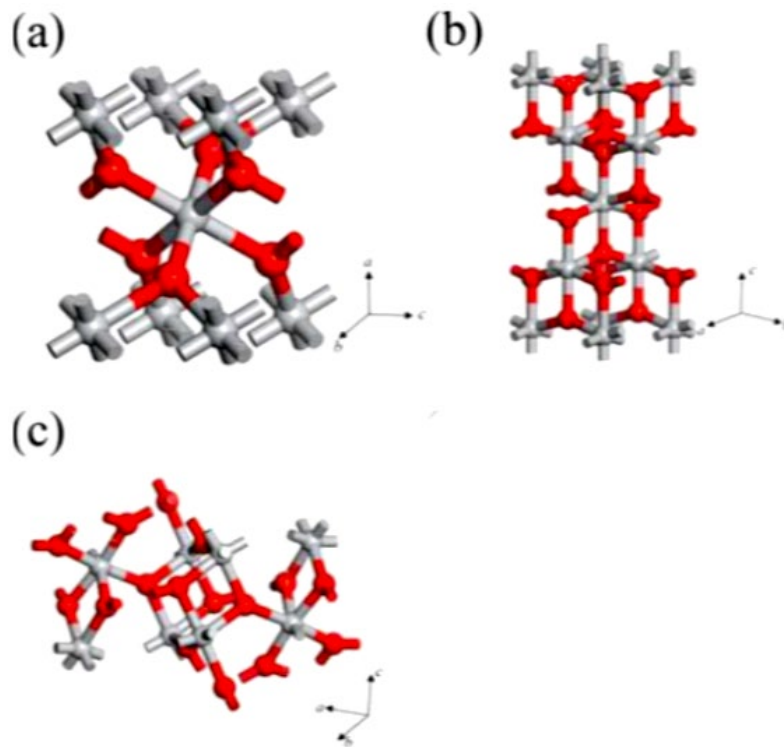


Figure1. 1: Crystal structures of TiO₂: (a) Rutile; (b) Anatase; (c) Brookite, red spheres represent Ti atoms, and the grey spheres represent O atoms. [15].

Figure1.1 represents the crystal structures of titanium dioxide (TiO₂) phases. Brookite (orthorhombic crystal system) has a band gap of 2.96 eV, while anatase (tetragonal system) has a band gap of about 3.2 eV. Rutile (tetragonal system) exhibits a band gap of 3.02 eV [15].

2.1.1 Preparation methods of titanium dioxide (TiO₂) thin films

Titanium dioxide (TiO₂) thin films, used in this study, are considered to be of great importance due to their wide range of applications in various fields, such as biotechnology, tribology, and optical applications [16].

There are a lot of techniques used to prepare the porous thin films of TiO₂ including chemical vapor deposition (CVD) and physical vapor deposition (PVD) processes.

- **Chemical vapor deposition (CVD)**

Chemical vapor deposition (CVD) is a process used to prepare thin films on the surface of a solid material starting from gases that contain the elements to be deposited. The process involves the decomposition of these gases, which are then converted into solid materials that deposit on the surface, forming a substrate layer. CVD includes several methods such as: low pressure chemical vapor deposition (LPCVD), plasma enhanced chemical vapor deposition (PECVD), ...

- **Physical vapor deposition (PVD)**

Physical vapor deposition (PVD) is used for the preparation of thin films by vaporizing a material in either its solid or liquid state. the vapor then condenses on the substrate surface to form a thin layer. this process is carried out under vacuum, low-pressure gas, or plasma conditions to facilitate the transfer of atoms and reduce contamination. the deposition rate generally ranges from 1 to 10 nm per second, which allows good control over the thickness of the deposited layer [17]. This process contains several techniques like: sputtering, thermal evaporation, ...

- **Drop casting method**

In the drop-casting technique, a small volume of a prepared substance (water plus TiO₂ powder) is deposited directly onto a clean solid substrate, where the solution spreads over the surface and the final structure is formed during the drying process. This process involves a set of simultaneous physicochemical phenomena, including droplet spreading on the surface, evaporation of the solvent, nucleation and growth of water droplets, followed by their coalescence and self-organization. after that, water evaporation and finally substance solidification, leading to the formation of a porous film [18] .

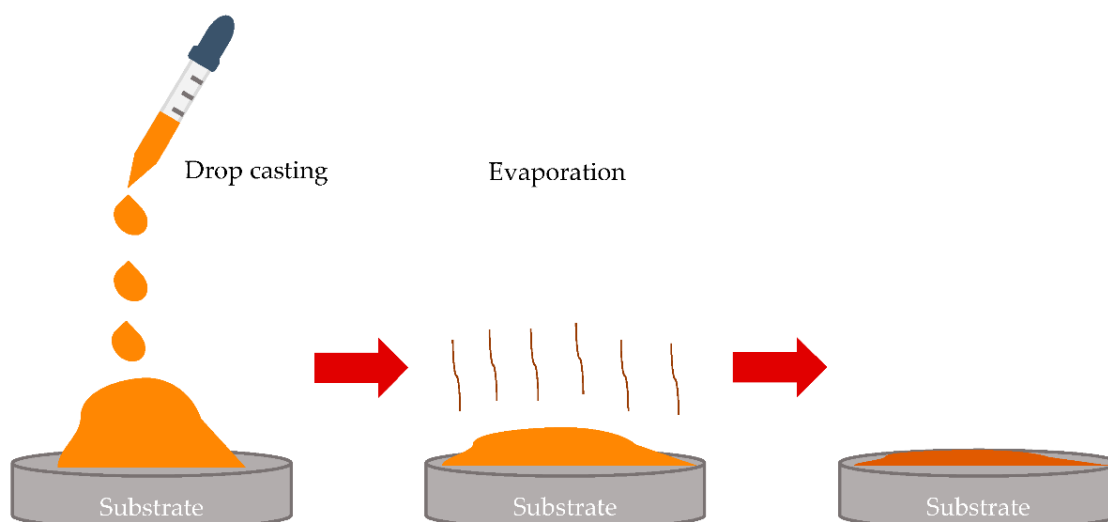


Figure1. 2: Scheme representation of the drop-casting process. [18].

3 Proteins

Proteins are biopolymers in which amino acids with different physicochemical properties are polymerized. the physicochemical and biochemical properties of individual proteins differ depending on their constituent amino acids. physicochemical and biochemical properties of individual proteins can be measured using various experimental methods, although such measurements are generally laborious. when analyzing proteins, it would be very useful if their properties were clarified to some degree beforehand. Various in silico methods and tools used for predicting or calculating various protein properties such as molecular weight, isoelectric point, solubility, crystallizability, and hydrophobicity have been widely discussed and presented.

There are many types of proteins commonly used in research, including Fibronectin (FN), Human Serum Albumin (HSA), and Bovine Serum Albumin (BSA) [19]. This latter (BSA), was selected, in this study, as the model protein due to its wide use in biointerface and biomaterials research, as well as its well-known structure and stability properties.

3.1 Bovine Serum Albumin (BSA)

Bovine serum albumin (BSA) is a globular protein derived from bovine serum and consists of 583 amino acid residues [5]. It has a molecular weight of 66.43 kDa (66430 g/mol). Its primary structure is characterized by a heart-like shape, while its secondary structure consists of 50–68% α -helices and 16–18% β -sheets [8]. In addition, this protein has several advantages over other therapeutic proteins in terms of stability, availability, and cost.

Therefore, it is widely used as a model protein to investigate the properties and performance of electrospun fibers for protein encapsulation [5].

BSA adopts an elongated ellipsoidal shape with approximate dimensions of $4 \times 4 \times 14$ nm³. It also exhibits a flexible structure that can change depending on the pH value. Its forms are classified into three states: the native (N) form, which exists in the pH range of 8 to 4.3; the fast-migrating (F) form, which appears at pH values below 4.3 down to 2.7; and the transition from the F form to the E form at pH values below 2.7, which is accompanied by further protein expansion and a significant increase in intrinsic viscosity [1]. As well as albumin has an important functional role represented in maintaining the hydrogen ion concentration in the blood due to its ability to bind with acids and bases, and transport minerals, fatty acids, cholesterol, bile acids, hormones and drugs, as well as maintaining the osmotic pressure inside blood vessels, which is necessary for delivering oxygen and blood to tissues [20].

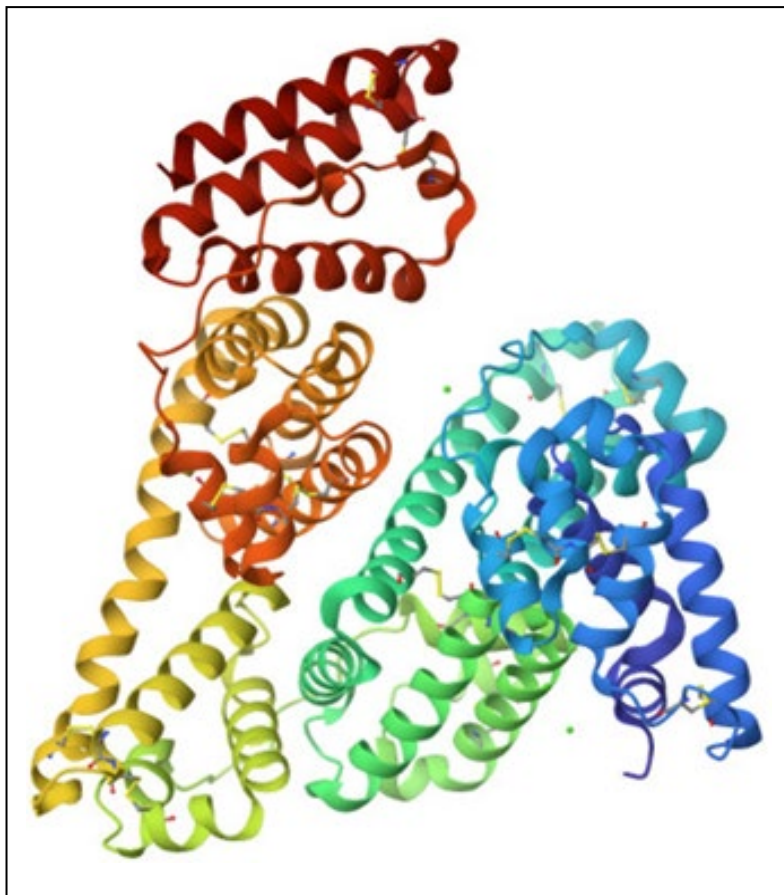


Figure1. 3: Three dimensional Structure of bovine serum albumin (BSA) (PDB ID-4F5S). [21].

CHAPTER 1 : Surface – Protein interactions

Table 1.1 bellow presents the amino acid composition of BSA, from which the elemental composition of the protein can be deduced through the assembly of these amino acids.

Table 1. 1: The amino acids composition of BSA protein (*presence of S in the molecule) . [8].

Amino Acids	Number of residues	
Aspartic acid	41	41
Arginine	23	26
Asparagine	13	14
Glutamic acid	59	58
Glutamine	20	21
Histidine	17	16
Lysine	59	60
Serine	28	32
Threonine	34	34
Alanine	46	48
Isoleucine	14	15
Leucine	61	65
Methionine*	04	05
Phenylalanine	27	30
Tryptophan	02	03
Tyrosine	19	21
Valine	36	38
Cysteine*	35	35
Glycine	16	17
Proline	28	28
Total	582	607

(Brown et al. 1975) (Hirayama et al. 1990)

Table 1. 2: The BSA atomic composition indicating the number of different atoms. [8].

Number of atoms	C	N	O	S
Brown et al [22]	2926	779	897	39
Hirayama et al [23]	3030	841	947	40

CHAPTER 1 : Surface – Protein interactions

Table 1.2 also shows the number of carbon, nitrogen, oxygen, and sulfur atoms that constitute the bovine serum albumin molecule according to two different references indicated in the tables.

4 Germanium (Ge) and Silicon (Si)

Germanium is an intrinsic semiconductor has a diamond crystallographic structure and possesses an indirect energy gap between the valence band and the conduction band. Its performance efficiency decreases at high temperatures; therefore, it has been replaced by silicon in many electronic applications and is now mainly used in the fields of optical fibers and infrared optics [24].

Germanium has an energy band gap of around 0.67 eV at room temperature, which allows it to exhibit excellent electrical conductivity. It is also characterized by spectral sensitivity at a wavelength of approximately (1.55 μm). It is characterized by high brittleness, and its hardness is 6.0 on the Mohs scale [25].

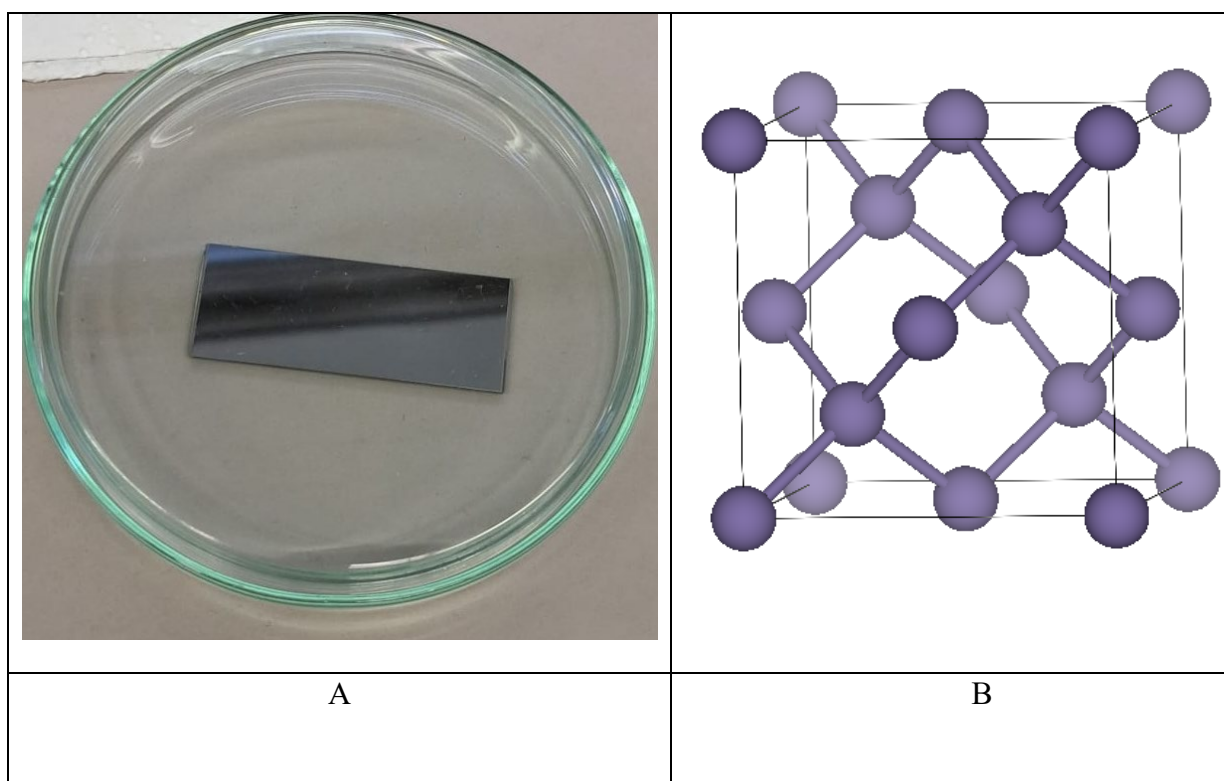


Figure1. 4: Germanium substrate (the ATR-IR) element) and B) Cristal structure of Germanium [24].

CHAPTER 1 : Surface – Protein interactions

Silicon is a semiconductor with an energy band gap of 1.12 eV. It is considered a fundamental material in the field of semiconductors due to its wide use in the electronics industry [8]. Both materials Si and Ge have similar structure and they are widely used in the field of optoelectronics applications.

5 Protein adsorption

It is the adhesion (linkage or sticking together) of proteins to a solid surface when they come into contact where some changes, upon adsorption, in the shape and structure of the protein are possible. This phenomenon is important for understanding and controlling the interactions between surfaces and biomolecules in biological and technological systems. It has also attracted considerable research interest in various fields, including medicine, pharmaceutical sciences, analytical sciences, biotechnology, cell biology, and biophysics. When a protein approaches a surface, its free energy balance changes as it enters a different environment. This difference drives the protein to reorganize its structure in order to become more stable on the surface, leading to an increase in the contact area with the surface.

Protein adsorption is considered a complex phenomenon governed by several driving forces, such as electrostatic repulsion and attraction, depending on the charges of both the protein and the surface, which determine its approach to the surface and its orientation upon adsorption. Hydrogen bonds also contribute to stabilizing the adsorbed protein, while van der Waals forces enhance the binding interaction, in addition to hydrophobic and hydrophilic interactions.

External environmental conditions also affect protein adsorption, as pH determines the protein's charge. At the isoelectric point, the net charge of the protein becomes zero, leading to a reduction in electrostatic repulsion, which in turn results in an increase in adsorption. Temperature also increases the affinity for surface binding by affecting the mobility and structural stability of the protein. Ionic strength, which is related to the Debye length that expresses the extent of the decay of the electric potential of a fixed charge in an electrolytic medium, when it increases, this is accompanied by a decrease in the range of electrostatic interactions, i.e., a decrease in the electrical interactions between molecules [1].

5.1 Steps of protein adsorption

The adsorption process proceeds through several fundamental stages, namely:

5.1.1 Transport of proteins from the bulk solution phase towards the interfacial region of the surface

Protein molecules are transported from the liquid medium toward the solid interface as a result of the random thermal motion of molecules and diffusion caused by the concentration gradient between the solution and the surface. The rate of this process is influenced by several factors, including the molecular weight of the protein, its structural dimensions, its ionic nature, as well as environmental conditions such as temperature and concentration.

5.1.2 Initial attachment of protein to the surface:

Protein molecules adhere to the solid surface for a period of time sufficient to initiate the adsorption process, where the strength of the interaction between the protein and the surface is the main factor determining how long the molecules remain bound during this stage. where adsorption mechanisms can vary, such as random attachment to active sites, ordered adsorption, or the formation of protein clusters on the surface.

5.1.3 Conformational changes of proteins on the surface

In this stage, the conformational structure of adsorbed proteins may change during the adsorption process or due to environmental factors such as temperature and the pH of the solution. These changes can increase their residence time on the surface and strengthen their binding, as a result of modifications in the interaction energy between the proteins and the surface

5.1.4 The detachment of adsorbed protein from the surface

Protein molecules desorb from the surface when the interaction force between them and the surface is weak, as in physical adsorption. In this case, adsorption can be partially reversible due to conformational changes that occur during adsorption and the ability of the protein to bind to the surface through multiple regions of its molecular structure.

5.1.5 Transport of proteins from the surface:

Detached protein molecules leave the surface and move into the surrounding medium with a modified structure compared to their original native state. They may also re-adsorb onto the surface again under certain conditions. After that, proteins interact with the surface electrostatically through van der Waals forces and hydrophobic interactions, until a state of

equilibrium is reached, leading to system stability and the completion of the protein adsorption process [8].

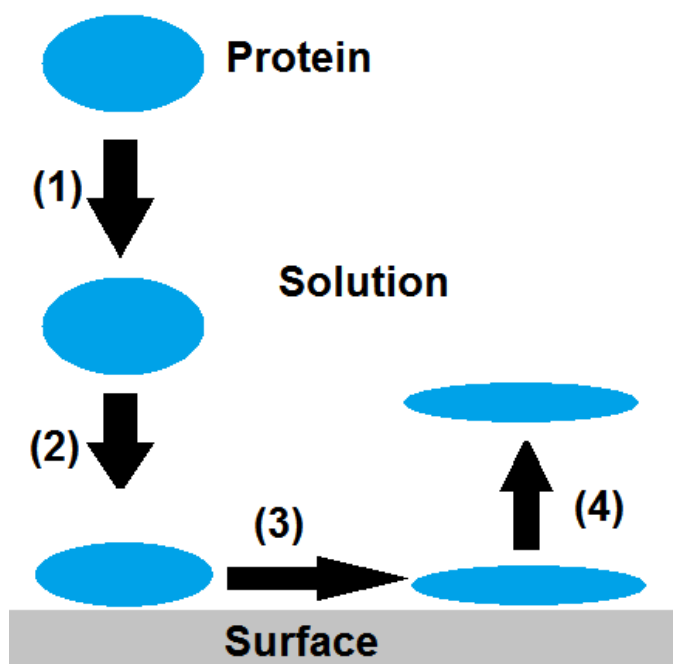


Figure1. 5: Basic steps of protein adsorption on a solid surface. notice the shape modification of the molecules as they interact with the surface. [8].

In this study, the focus is on the adsorption of bovine serum albumin (BSA) onto a porous titanium dioxide surface, using a germanium substrate. This phenomenon used to understand protein–solid surface interactions and the mechanisms of binding.

Protein adsorption can be studied using theoretical models to describe the kinetics, and numerical simulations can also be used to understand the behavior at the molecular level. Theoretically, adsorption is often described using models such as the Langmuir isotherm, although more complex models are required to take into account protein structural changes and different interactions. Experimentally, techniques such as FTIR-ATR allow in situ analysis of adsorption behavior, while microscopy techniques provide information about surface morphology.

To understand the mechanisms of protein adsorption and the interactions at the interface between the solid surface and the protein, appropriate analytical techniques are used. These techniques allow obtaining important information about the adsorption process, such as the amount of adsorbed protein, the structural changes it undergoes, and the nature of the interactions between the protein and the surface.

There are several experimental methods for studying protein adsorption, including spectroscopic techniques, microscopy techniques, as well as surface-sensitive techniques and infrared spectroscopy. In this context, Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) is widely used as an effective and non-destructive technique, allowing the in situ study of protein adsorption on solid surfaces.

6 FTIR-ATR spectroscopy:

Fourier Transform Infrared (FTIR) spectroscopy is considered one of the most important analytical techniques used to study the chemical structure of materials. It works by exposing the sample to infrared radiation, which interacts with the chemical bonds within the molecule. This technique measures the amount of absorption resulting from the vibrations of these bonds, leading to a characteristic spectrum that is typically presented as a relationship between transmittance or absorbance and wavenumber (cm^{-1}). This allows for the accurate determination of the chemical composition of the sample. This technique is considered important in the study of protein adsorption, as it allows the analysis of structural changes and the deduction of adsorption mechanisms or sites based on spectroscopic indicators, such as the changes that occur in the Amide I and Amide II bands, in addition to the appearance or disappearance of new peaks in the spectrum. In the FTIR spectrum of proteins, two main bands are observed, known as Amide I in the range of $1600\text{--}1700\text{ cm}^{-1}$ and Amide II ($1500\text{--}1600\text{ cm}^{-1}$), which are associated with the characteristic vibrations of peptide bonds within the protein. Amide I, which results from the stretching vibration of the C=O bond present in the peptide bonds of the protein, represents the most intense band in the spectrum. In contrast, Amide II arises from the bending vibration of the N–H bond and the stretching vibration of the C–N bond. These two bands are used to monitor the structural changes that occur in the protein before and after the adsorption process [6].

The secondary structure of proteins was analyzed using the Amide I band. There are two main approaches for this analysis: where deconvolution techniques of the Amide I band such as second derivative and FSD are used to resolve the overlapped peaks in the band and identify the peaks and their positions, followed by curve fitting of the band, which is considered the next step in the analysis of FTIR spectra, used for the quantitative analysis of protein secondary structures within the Amide I region [26].

The bands in the range of $1650\text{--}1658\text{ cm}^{-1}$ are associated with the α -helix structure, while the bands in the range of $1620\text{--}1640\text{ cm}^{-1}$ are associated with the β -sheet structure. Variations in hydrogen bonding strength also affect the positions of these bands, leading to

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shifts in their locations within the spectrum, where β -turn bands are typically found in the range of 1660–1690 cm^{-1} , as shown in Table 3 [6].

Table 1. 3: Assignment of bands associated with secondary components of amide I. [8].

2nd structure components	Wavenumbers (cm^{-1})	
	Average	Extremes
β -sheet	1633	1623-1641
α -helix	1654	1648-1657
β -turn	1672	1662-1686

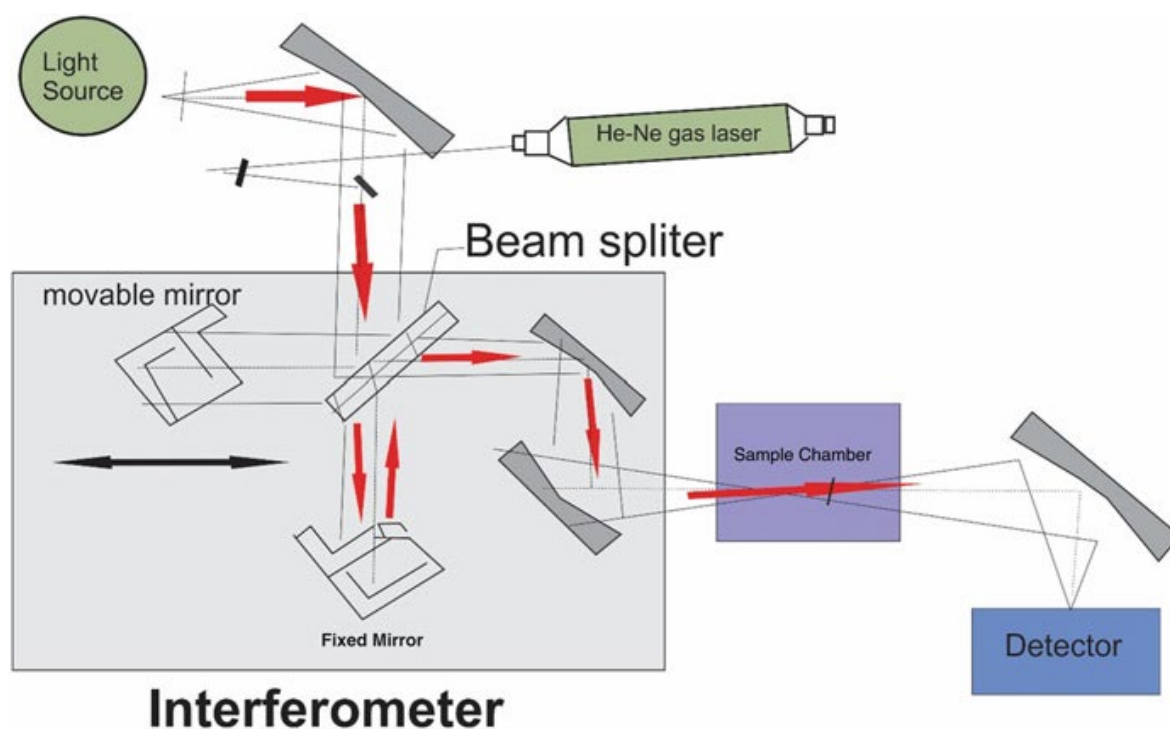


Figure1. 6: Instrumentation of the double-beam (top) and single-beam (bottom) IR spectrophotometers. [6].

6.1 Historical overview

Sir Frederick William Herschel was the first to discover infrared radiation through an experiment in which he used mercury-in-glass thermometers placed under sunlight that had been passed through a glass prism (1800). He observed that heat was not limited to the red region of the visible spectrum, but rather that the highest intensity of heat was found beyond the visible range. Infrared spectroscopy was further developed effectively for practical spectral measurements. Sir John Frederick William Herschel, the son of Herschel, was among the scientists who contributed to this development.

Rubens and Wood carried out important work on spectral interference, as did Rubens and von Baeyer in the field of spectroscopy, prior to the development of the Michelson interferometer.

In the 1960s, techniques were developed that played a role in improving the signal-to-noise ratio. In the late 1970s, infrared microscope systems appeared on the market. In the early 1980s, significant progress was achieved with the availability of high-resolution instruments that were commercially accessible, possessing a maximum optical path difference of 250 cm^{-1} , associated with an unapodized resolution of $\tilde{\nu} = 0.61\text{ d}^{-1}\text{MOPD} = 0.0024\text{ cm}^{-1}$.

6.2 Applications of FTIR Spectroscopy

FTIR is considered an effective analytical technique used to study a wide range of samples across various fields.

- Qualitative and quantitative analysis.
- Study of polymers and their degree of crystallinity.
- Detection of organic contaminants on surfaces.
- Analysis of thin films and adhesive materials.
- Investigation of surface defects.

7 Kinetic models

Kinetic models of BSA adsorption are mathematical tools that help us understand and simulate how the protein adsorption process evolves over time on a surface, taking into account all interactions and structural changes. Several models have been formulated to describe the kinetics of protein adsorption:

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7.1 Pseudo-first Order (PFO)

It is used to describe adsorption processes controlled by surface interactions. The pseudo-first-order model is given by:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (1.1)$$

k_1 : first-order adsorption rate constant

q_e : amount of adsorbed substance at equilibrium

t : adsorption time

q_t : amount of adsorbed substance at time t

When integrated for boundary conditions $t = 0$ to t and $q_t = 0$ to q_t , the equation becomes:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (1.2)$$

7.2 Pseudo-Second Order (PSO)

The pseudo-second-order model assumes that adsorption is governed by surface control, and its linear form is expressed as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (1.3)$$

k_2 : second-order adsorption rate constant

q_e : amount of adsorbed substance at equilibrium

t : adsorption time

q_t : amount of adsorbed substance at time t

Upon rearrangement and integration for boundary conditions $t = 0$ to t and $q_t = 0$ to q_t , the PSO equation can be expressed as:

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \quad (1.4)$$

7.3 Elovich model

The Elovich model describes adsorption on energetically heterogeneous surface sites have the following form:

$$q_t = 1/\beta \ln(1 + \alpha \beta t) \quad (1.5)$$

t : adsorption time

q_t : amount of adsorbed substance at time t

α : initial adsorption rate

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β : an empirical constant connected with the activation energy distribution.^{37,40} When integrated and assuming $\alpha\beta t \gg 1$

When integrated and assuming $\alpha\beta t \gg 1$, this equation can be simplified to:

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t) \quad (1.6)$$

7.4 The intraparticle diffusion model

This model describes the case in which the adsorption rate is limited by the diffusion process within the pores. It also differs from other kinetic models that depend on energy control related to the surface of the adsorbent. Its equation is written in the following form:

$$qt = k_d \sqrt{t} + C \quad (1.6)$$

q_t : amount of adsorbed substance at time t

k_d : the intraparticle diffusion rate constant.

C : intercept related to the boundary layer (film diffusion) effect

t : adsorption time [27].

Model parameters were derived from rate plots using linear regression in Microsoft Excel. Models fit with these parameters were compared with experimental data by determining the root-meansquare error (RMSE) of the respective models with the following equation:

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (q_{t_{obs}} - q_{t_{pred}})^2} \quad (1.7)$$

8 Comparison between kinetic models

The Pseudo-first-order model is suitable for describing the initial stages of adsorption on a homogeneous surface and weak interaction systems. The Pseudo-second-order model is generally the most suitable for protein adsorption systems on heterogeneous surfaces with complex interactions. It reflects strong electrostatic interactions, multi-point binding between the protein and the surface, as well as possible conformational rearrangements of the protein structure during adsorption. Elovich describes complex surfaces and protein rearrangement on a heterogeneous surface, where the adsorption rate decreases over time due to an increase in activation energy.

Conclusion

The study of protein adsorption on titanium dioxide surfaces is based on understanding the protein–surface interactions and the influence of the surrounding environment. The kinetic models help to understand the mechanisms and rate of adsorption. FTIR-ATR spectroscopy analytical technique is effective and accurate tools for measuring the amount of adsorption, as well as the structure and interactions involved. Kinetics models are of a great importance to investigate the protein adsorption where the Pseudo-second-order (PSO) model stated as the most widely used and suitable for describing the kinetics of protein adsorption on porous surfaces, compared to the Pseudo-first-order (PFO) and Elovich models. In the next following part of this manuscript, we are going to discuss in more details a proposed three state kinetics model that will be used to study the adsorption of BSA on different surfaces (Ge, Si and porous TiO₂) taking into account the pores included in the thin film in order to determine their effect on the adsorption.

CHAPTER 2

BSA adsorption on porous TiO₂ films:

***Kinetics study investigated by three
states model***

CHAPTER 2: BSA adsorption on porous TiO₂ Films - Kinetics study investigated by three states model -

1 Introduction

In this chapter, the adsorption of bovine serum albumin (BSA) protein was investigated on different surfaces including silicon and germanium, which are considered among the most important materials used in surface science [22]. Adsorption was also studied on a porous titanium dioxide (TiO₂) surface and on a germanium substrate. Fourier Transform Infrared Spectroscopy (FTIR) technique was used to monitor the adsorption process and analyze the changes. Several kinetic models were used, including the pseudo-first-order (PFO) model and the pseudo-second-order (PSO) model, which describe adsorption kinetics when the adsorption rate is limited by interfacial energetic barriers on the surface, in addition to the Elovich model, which is used to describe surface-controlled adsorption on energetically heterogeneous surfaces, as well as the simplified intraparticle diffusion model to describe the adsorption rate limited by the diffusion of molecules within pores [25]. Although these classical models are widely used to describe adsorption kinetics, they cannot distinguish between surface adsorption and the transport of protein molecules inside the pores, that is, they provide only a partial description. Since protein adsorption does not occur only on the external surface but also extends into the pores, this makes the adsorption mechanism complex and multistep, leading to the need to develop a more comprehensive kinetic model to represent all surface adsorption mechanisms and diffusion within the porous structure in an accurate and realistic manner.

This chapter presents the experimental methodology and the application of both classical kinetic models and the proposed model to analyze the adsorption behavior of BSA protein on different surfaces.

2 Preparation of TiO₂ thin films

Experiments were carried out using commercial titanium dioxide (TiO₂) anatase nanoparticles purchased from Sigma-Aldrich, with an average particle size of less than 25 nm, a specific surface area ranging from 200 to 220 m²/g, and a density of 3.9 g/cm³ at 25 °C. To prepare the porous films, 20 mg of TiO₂ powder were dispersed in 10 mL of purified Milli-Q water (18 MΩ·cm), followed by sonication for 30 minutes in order to obtain a homogeneous and stable suspension. The suspension was then deposited onto a germanium internal reflection element (Ge IRE) with dimensions of (52 × 20 × 1 mm³). Prior to deposition, the substrate surface was cleaned using ethanol and subsequently treated with air

CHAPTER 2: BSA adsorption on porous TiO₂ Films - Kinetics study investigated by three states model -

plasma for approximately 15 minutes in order to remove impurities and improve surface cleanliness [7].

TiO₂ thin films were prepared using the drop-casting technique through several steps. In the first stage, a small amount of titanium dioxide suspension was dropped onto the surface of the substrate using a micropipette, and then left to spread uniformly over the surface. This was followed by the drying stage, where the coated substrate was heated at approximately 35 °C to evaporate the remaining solvent. This process was repeated three times in order to increase the film thickness and improve its homogeneity. After that, the films were dried in an oven at approximately 80 °C for 10 min. Finally, after thermal treatment, a stable TiO₂ thin film was obtained, ready for adsorption experiments [28].



Figure2. 1: Porous thin film of TiO₂ prepared by drop casting on Ge ATR element.

3 The BSA adsorption experimental setup

A water-based BSA solution at a concentration of 10^{-6} mol/L was prepared for use in the adsorption experiments. In situ attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was utilized to monitor BSA adsorption in real time on the three surfaces of interest. The experimental configuration is schematically represented in Figure 2.2 The BSA solution was continuously circulated over the sample surface via a peristaltic pump connected to the inlet of the flow-through sample holder, enabling direct contact between the protein solution and the adsorbent surface. Sequential spectra were acquired over a period of 90 minutes, with a 2-minute interval between successive measurements.

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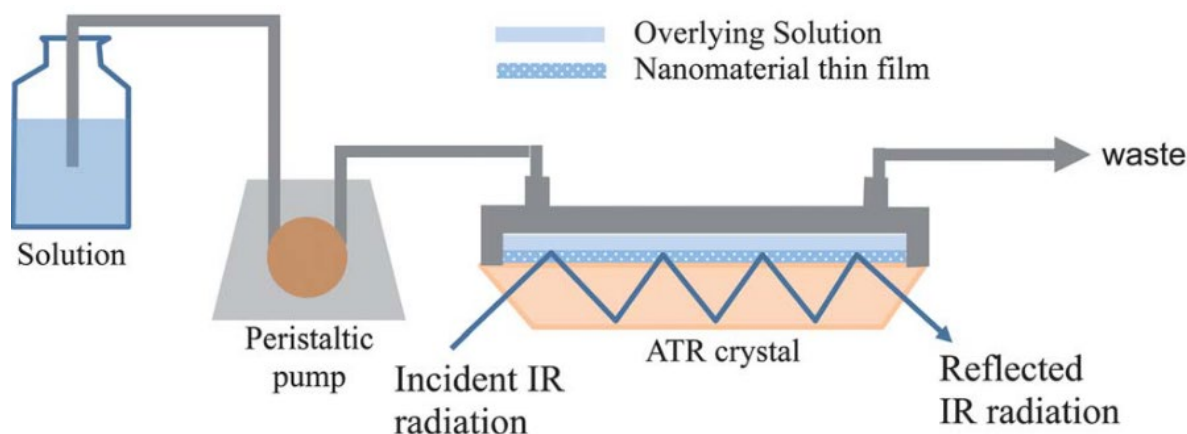


Figure2. 2: Schematic illustration of the in situ ATR-FTIR flow-through experimental setup employed for real-time monitoring of BSA adsorption at the liquid–solid interface. [29].

4 Three states kinetics models

The three states referred to in this model correspond to: the protein molecules in the bulk solution (b state), the molecules in the diffusion in the film pores (d state), and the adsorbed molecules (“as” state for surface adsorption and “ap” state for pore adsorption). These states are interconnected through a set of kinetic rate constants. Specifically, K_{asb} represents the surface desorption rate constant, K_{db} the diffusion-to-bulk transfer rate constant, K_{bd} the bulk-to-diffusion transfer rate constant, K_{dap} the surface adsorption rate constant, K_{apd} the pore adsorption rate constant, and K_{pad} the pore desorption rate constant. Together, these constants describe the dynamic exchange of protein molecules between the bulk solution, the diffusion state, and the adsorption sites on the porous surface. It should be noted that once a protein molecule is adsorbed onto the surface, it cannot subsequently be adsorbed within the pores, and vice versa. In other words, a molecule adsorbed on the external surface remains in the surface-adsorbed state, while a molecule adsorbed within the pores remains in the pore-adsorbed state. Consequently, no direct exchange of molecules occurs between the surface-adsorbed state (as) and the pore-adsorbed state (ap). This assumption explains the absence of any kinetic rate constant connecting these two adsorption states in the proposed model.

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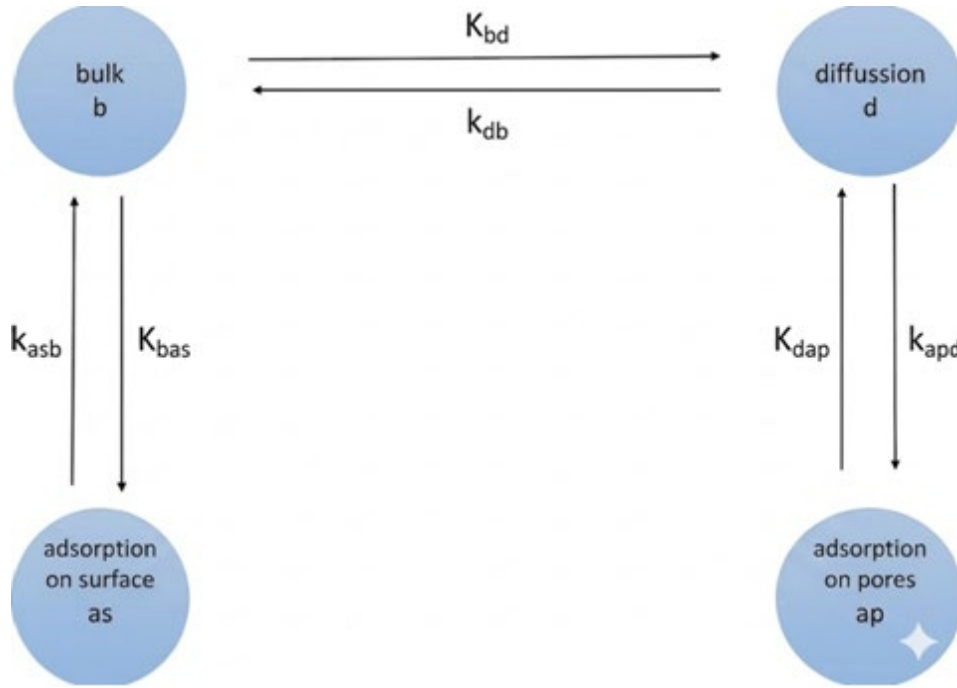


Figure2. 3: Three-State Kinetic Diagram for Bovine Serum Albumin (BSA) Adsorption.

Based on previous build kinetics models such as the two state model reported by Hu et al. [30], the variation as a function of time of the number of proteins molecules in different states is given by the following set of coupled differential equations

$$\frac{\partial}{\partial t} Nb(t) = K_{asb} \cdot Nas(t) + K_{db} \cdot Nd(t) - K_{bas} \cdot Nb(t) - K_{bd} \cdot Nb(t) \quad (2.1)$$

$$\frac{\partial}{\partial t} Nas(t) = K_{bas} \cdot Nb(t) - K_{asb} \cdot Nas(t) \quad (2.2)$$

$$\frac{\partial}{\partial t} Nap(t) = K_{dap} \cdot Nd(t) - K_{apd} \cdot Nap(t) \quad (2.3)$$

$$\frac{\partial}{\partial t} Nd(t) = K_{bd} \cdot Nb(t) + K_{apd} \cdot Nd(t) - K_{db} \cdot Nd(t) - K_{dap} \cdot Nd(t) \quad (2.4)$$

Where number of BSA proteins in bulk, surface, pores and diffusion states is named

$Nb(t)$, $Nas(t)$, $Nap(t)$ and $Nd(t)$ respectively.

It was clearly demonstrated previously [31] that BSA proteins irreversibly adsorb on the germanium, silicon and TiO₂ surfaces. Therefore, K_{asb} , K_{apd} are zero consequently, the previous system of equation become:

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$$\frac{\partial}{\partial t} Nb(t) = Kdb \cdot Nd(t) - Kbas \cdot Nb(t) - Kbd \cdot Nb(t) \quad (2.5)$$

$$\frac{d}{dt} Nas(t) = Kbas \cdot Nb(t) \quad (2.6)$$

$$\frac{\partial}{\partial t} Nap(t) = Kdap \cdot Nd(t) \quad (2.7)$$

$$\frac{d}{dt} Nd(t) = Kbd \cdot Nb(t) - Kdb \cdot Nd(t) - Kdap \cdot Nd(t) \quad (2.8)$$

5 Data analysis

This system was solved using MAPLE 17 software and the final solution was integrated in a python code presented in Anexe I along with the three other mentioned models (PFO, PSO, Intraparticle diffusion reported at the end of the previous chapter) to fit the experimental data that will be shown in the upcoming sections. The objective of this step was to determine the rate constants mentioned before in order to find the amount of BSA molecules in the different states. The quality fit was confirmed by residual graph that will be illustrated under its corresponding kinetics curve to compare the used four models as reported in the following findings section.

6 Results and discussion

Since PFO, PSO are signed to study surface-controlled adsorption while the intraparticle diffusion models was design to study the diffusion-controlled adsorption. The model proposed in the present work was developed to bridge this gap by simultaneously accounting for both surface- and diffusion-controlled adsorption mechanisms, which are expected to coexist within the context of porous film adsorption. To validate the proposed model; BSA adsorption was conducted on two types of surfaces including flat (silicon and germanium without pores) and porous TiO₂.

6.1 BSA on Silicon

Before the adsorption of the protein, the silicon surface was treated and cleaned to avoid any contamination. This surface is considered with neglected pores. Figure 2.4 depicts the major IR traces of BSA, amide I (1650 cm⁻¹) and amide II (1550 cm⁻¹). Amide I is corresponding the stretching vibration of C=O where amide II corresponds to H-N and C-N vibrations. It is known that amide I is used to study the conformational changes of the BSA molecules upon adsorption. The peak absorbance of each amide I band was plotted as a function of time and illustrated in figure 2.4 as a kinetic plot. The experimental kinetic data

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was fitted using the four models studied and the residual of each fit was plotted right below the kinetic plot on purpose to compare the models.

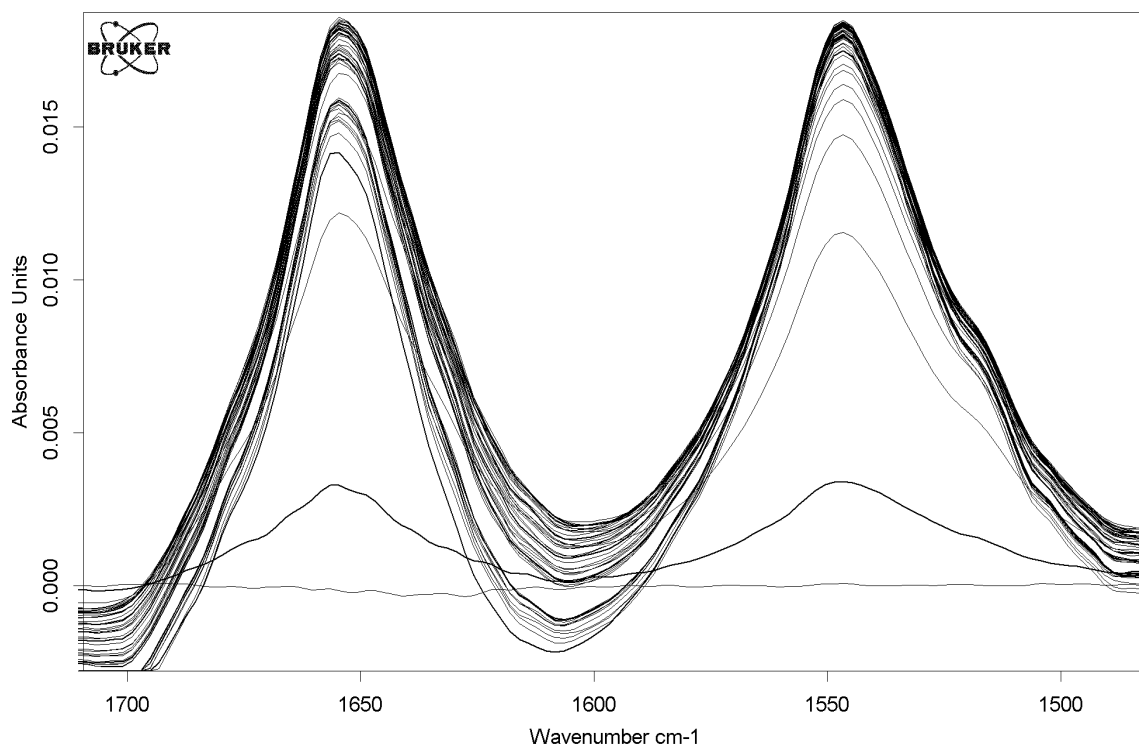


Figure2. 4: In situ ATR-FTIR measurements of BSA adsorption on Silicon surface, 47 s between two successive spectra.

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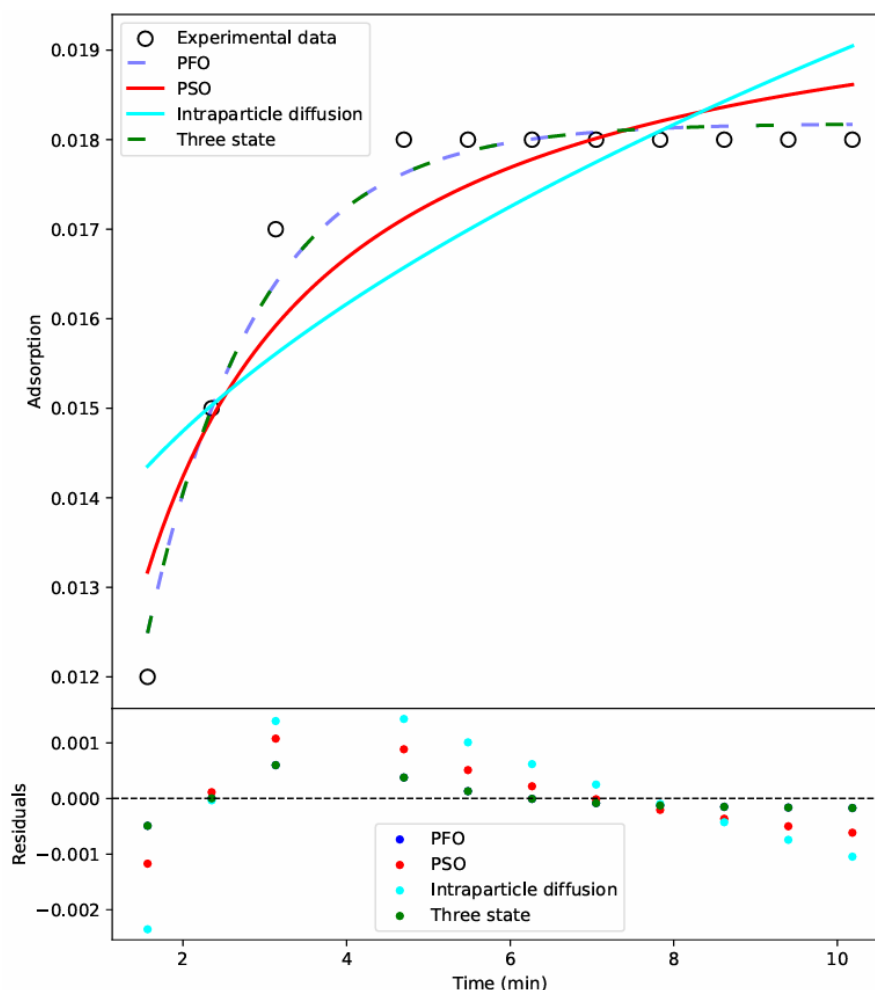


Figure2. 5: Fitting of experimental data using different kinetic models for BSA adsorption on silicon surface.

Figure 2.5 shows the fitting of experimental BSA adsorption kinetic data on the silicon surface using different kinetic models including the pseudo-first-order model PFO, the pseudo-second-order model PSO, the intraparticle diffusion model, and the three-state model. The results indicate that the experimental values increase rapidly in the initial stages, meaning that the adsorption rate is high at the beginning due to the availability of adsorption on non-porous surface leading to direct surface occupation on the material surface, then it gradually decreases to reach a state of stability (surface saturation) at point 0.018. This leads us to say that the studied adsorption process cannot be explained by a simple reaction, but rather involves several physical states (state in solution, diffusion and surface adsorption). The three-state model achieves the best agreement with the experimental data among all the

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models, as evidenced by its lowest residual values. This conclusion is further supported by the residual plot, where the residuals for the three-state model are distributed very close to zero, indicating a high goodness of fit. In contrast, the other models exhibit larger deviations from zero, suggesting that they do not fully capture the kinetic behavior of the system.

Table 2. 1: Kinetic parameters obtained from the fitting of BSA adsorption on the silicon surface data using different kinetic models. The k value is k_1 (min⁻¹), k_2 (min⁻¹) and k_d (a.u. min^{-1/2}) in case of PFO, PSO and intraparticle diffusion respectively. All the parameters of the three state model have the same unite (min⁻¹).

Model	Fitted Parameters	R ²	χ^2	reduced χ^2
Pseudo-First-Order (PFO)	$k_1 = 0.7415$	0.9767	9.0064	1.0007
Pseudo-Second-Order (PSO)	$k_2 = 60.0549$	0.8797	9.0008	1.0001
Intraparticle Diffusion (IPD)	$k_d = 0.0024$; $C = 0.0113$	0.6526	9.0000	1.0000
Three-State Model	$K_{bas} = 0.7415$; $K_{bd} = 0.0000$ $K_{dap} = 0.0010$; $K_{db} = 0.0013$	0.9767	7.0050	1.0007

Table 2.1 shows the kinetic values and statistical parameters resulting from the fitting of the experimental data of protein adsorption onto the silicon surface using the four models. Thus, by comparing the values given for each model, it appears that the three-state model is the most suitable and consistent with the experimental data compared to the other models, as it recorded the highest correlation coefficient, which is the closest to one ($R^2 = 0.9767$), along with the lowest value for the χ^2 error 7.0050, and this highlights that the mechanism of protein adsorption onto the silicon surface is mainly governed by surface interaction processes rather than diffusion-controlled mechanisms. While the pseudo-first-order model achieved an acceptable fit with a correlation coefficient estimated at ($R^2 = 0.9767$) with a χ^2

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error value of 9.0064, where it is considered, the best compared to the remaining models and the least compared to the previously mentioned model. The pseudo-second-order model showed a trend similar to the previous model but with relatively lower accuracy, where the coefficient of determination reached ($R^2 = 0.8797$), with a reduced χ^2 value of 1.0144. The intraparticle diffusion model recorded the lowest agreement compared to the previous models, with a correlation coefficient of ($R^2 = 0.6526$). However, this result confirms that diffusion is not the sole rate-limiting step, but rather a stage within the successive processes that govern the overall adsorption mechanism. Thus, it can be concluded that the three-state model is the most accurate and best suited for describing the kinetics of protein adsorption on the silicon surface, due to the contribution of multiple surface interaction and rearrangement processes.

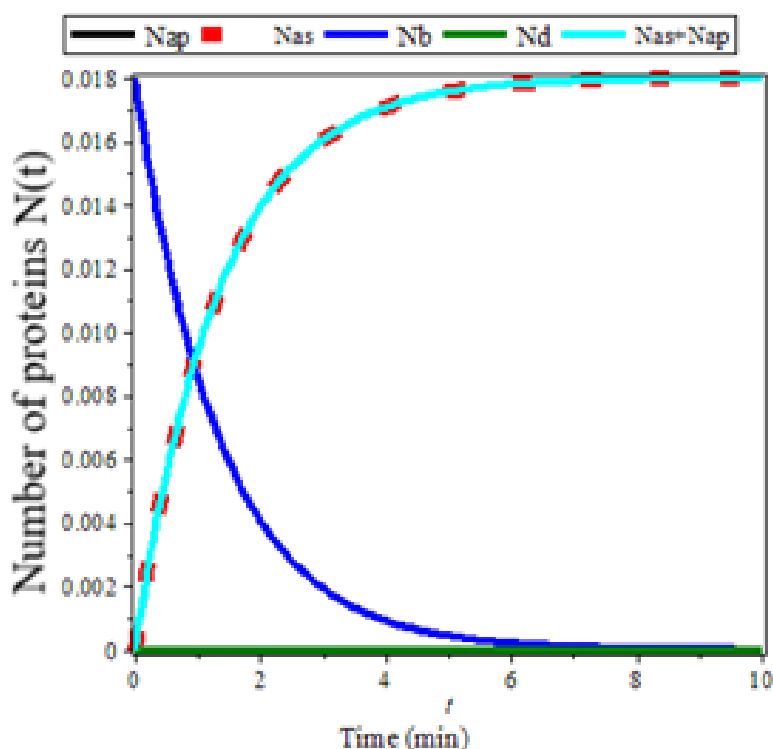


Figure2. 6: Time evolution of the protein population in the bulk phase, on the adsorbent surface, and within the pores

This curve represents the change in the amount of adsorbed BSA molecules on a silicon surface as a function of time. At the initial moment ($t = 0$), most of the molecules are present

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in the free state (N_b), while the populations of the other states are negligible, indicating minimal surface occupation. Subsequently, a sharp decrease in N_b is observed due to the rapid transfer of molecules from the bulk solution toward the solid surface. Concomitantly, N_{as} increases, indicating the progressive adsorption of molecules on the silicon surface.

While, N_{ap} is negligible in this case (non-porous surface), and N_d is nearly zero throughout the entire adsorption process. Thus ($N_{as} + N_{ap}$) is the same as N_{as} since the adsorption in the case is mainly on the surface as the pores in the silicon surface are negligible. This result gives a strong validation to the proposed model.

6.2 BSA on Ge

The germanium surface was similarly cleaned prior to experiments to ensure surface purity and eliminate possible contaminants. Figure 2.7 indicates the ATR-FTIR measurements where one can notice that the adsorption amount on the germanium is higher than the silicon one. To produce the kinetic plot the same method was used.

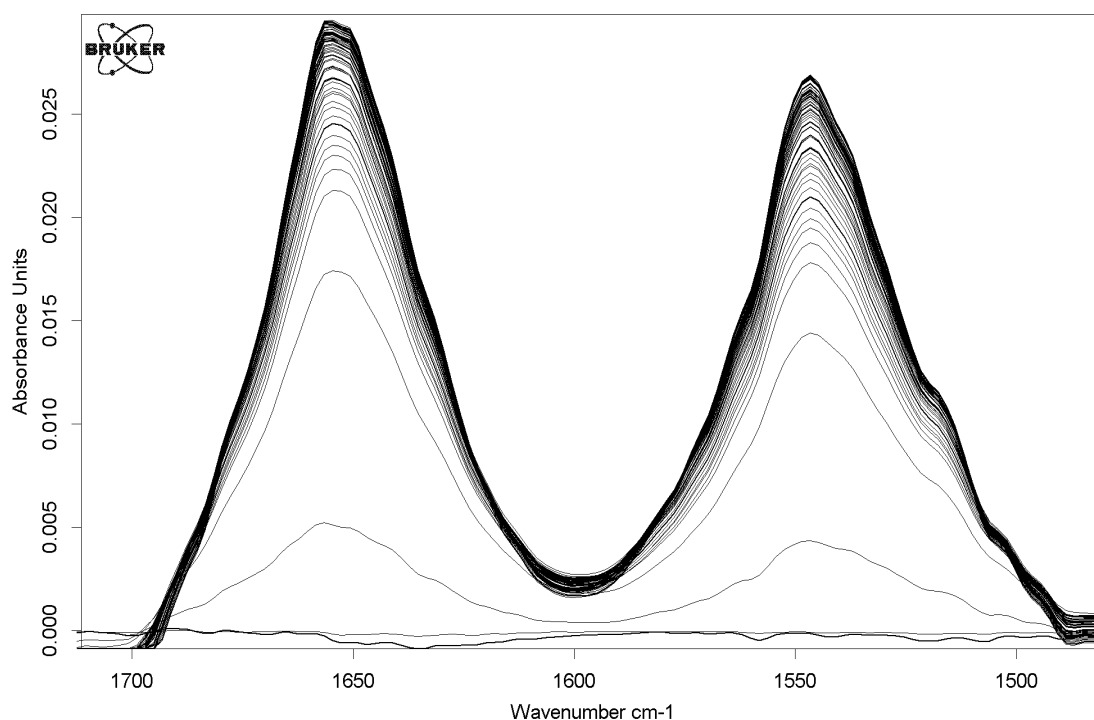


Figure2. 7: In situ ATR-FTIR measurements of BSA adsorption on Germanium surface, 47 s between two successive spectra.

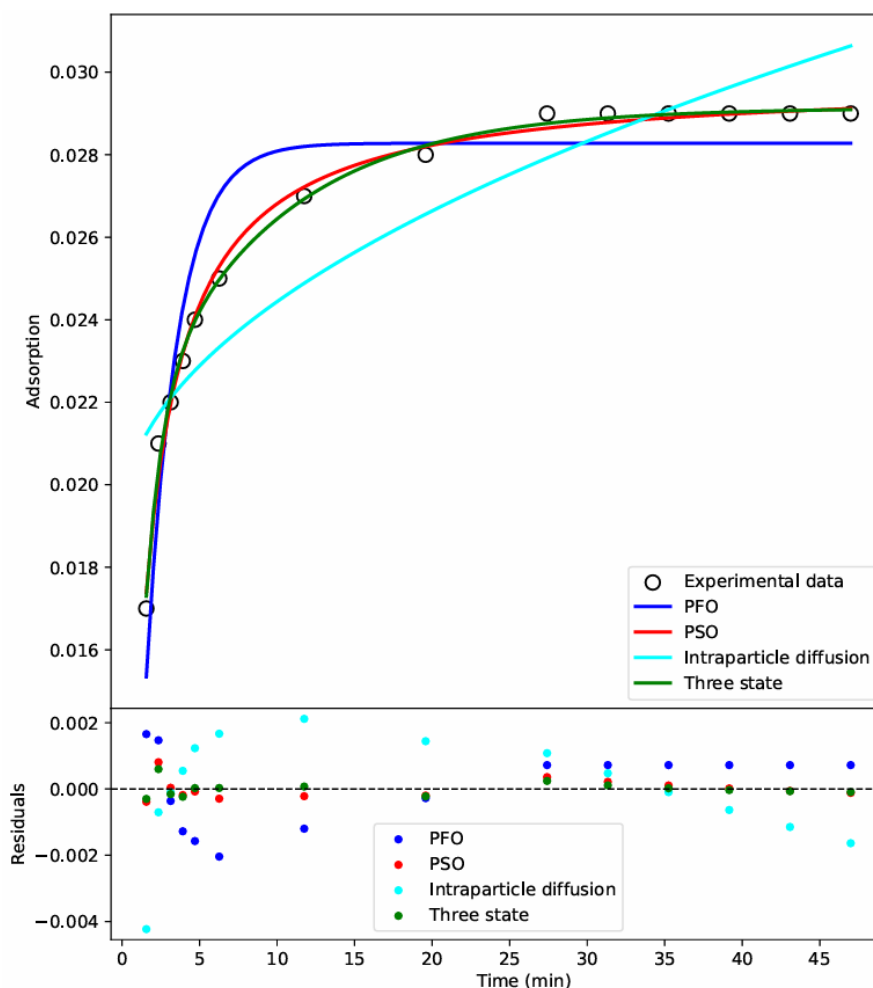


Figure2. 8: Fitting of experimental data using different kinetic models for BSA adsorption on the germanium surface.

Figure 2.8 represents the fitting of experimental BSA adsorption kinetic data on the germanium surface using different kinetic models: PFO, PSO, intraparticle diffusion model, and the three-state model. It is observed that the kinetics of the BSA adsorption on Germanium adopt a similar behavior as the silicon surface. The three-state model achieves the best agreement with the experimental data compared to the other models. This conclusion is further supported by the residual analysis, which shows that the residuals associated with the three-state model are closely clustered around zero, reflecting its superior goodness of fit. In contrast, the residuals of the other models display more pronounced deviations, indicating their inability to adequately describe the kinetic behavior of the system.

CHAPTER 2: BSA adsorption on porous TiO₂ Films - Kinetics study investigated by three states model -

Table 2. 2: Kinetic parameters obtained from the fitting of BSA adsorption on the germanium surface data using different kinetic models. The k value is k_1 (min⁻¹), k_2 (min⁻¹) and k_d (a.u. min^{-1/2}) in case of PFO, PSO and intraparticle diffusion respectively. All the parameters of the three state model have the same unite (min⁻¹).

Model	Fitted Parameters	R ²	χ^2	reduced χ^2
Pseudo-First-Order (PFO)	$k_1 = 0.4993$	0.9077	12.0242	1.0020
Pseudo-Second-Order (PSO)	$k_2 = 29.9607$	0.9937	12.0000	1.0000
Intraparticle Diffusion (IPD)	$k_d = 0.0017$; $C = 0.0191$	0.8180	12.0000	1.0000
Three-State Model	$K_{bas} = 0.6955$; $K_{bd} = 0.2287$ $K_{dap} = 0.1016$; $k_{db} = 0.0013$	0.9966	10.0003	1.0000

The kinetic analysis of protein adsorption on the germanium surface revealed that the three-state model provided the best agreement with the experimental data, exhibiting the highest correlation coefficient ($R^2 = 0.9966$) and the lowest χ^2 error 10.0003. This suggests that protein adsorption on germanium is a complex process involving multiple intermediate states rather than a simple adsorption mechanism. The pseudo-first-order and pseudo-second-order models showed acceptable fits but with lower accuracy than the three-state model. In contrast, the intraparticle diffusion model yielded the poorest agreement, indicating that diffusion has minimal effect within the overall adsorption process due to the smooth surface of the germanium. Therefore, the three-state model is the most suitable for describing the kinetics of protein adsorption on the germanium surface.

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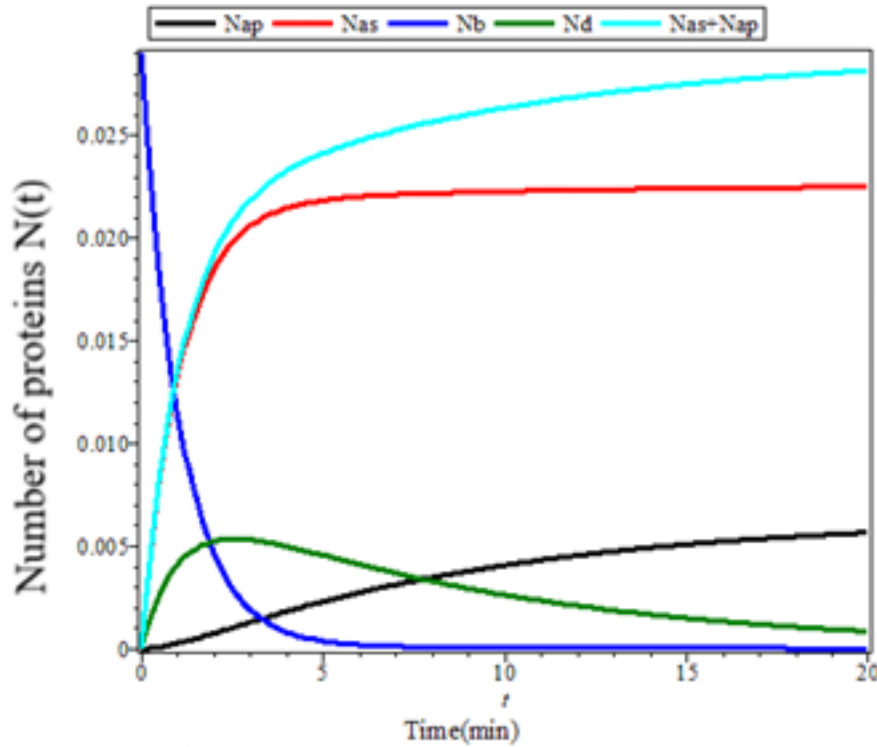


Figure2. 9: The curve represents the variation in the number of proteins in different states (bulk, surface adsorption, and pore adsorption) as a function of time

The curve represents the change in the amount of adsorbed BSA molecules on a germanium surface as a function of time at ($t = 0$), most of the molecules are present in the free state (N_b), then it decreases very rapidly over time as a result of their rapid transition toward the surface and the availability of adsorption sites. At the same time, we observe an increase in both surface adsorption (N_{as}) and surface diffusion (N_{ap}), where (N_{as}) increases more rapidly compared to (N_{ap}), reaching a quasi-steady state. This is attributed to the occurrence of surface adsorption first due to the direct accessibility of surface sites. In contrast, (N_{ap}) increases more slowly as a result of the longer time required for molecules to redistribute on the surface and reach the saturation state. As for adsorbed state on surface (N_d), it increases slightly at the beginning and then gradually decreases until it becomes nearly negligible, which reflects the short duration of the transitional phase between the bulk state and adsorption sites. The total adsorption ($N_{as} + N_{ap}$) shows a rapid increase at the beginning before approaching a steady state, which reflects the system's progression toward the saturation of adsorption sites.

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6.3 BSA on TiO_2/Ge

Following the preparation of the TiO_2 thin film on the cleaned germanium substrate, as detailed in the Experimental section, the sample was placed in the sample holder. Subsequently, the protein solution was introduced over the surface, and the adsorption of BSA was monitored in real time as indicated in figure 2.10.

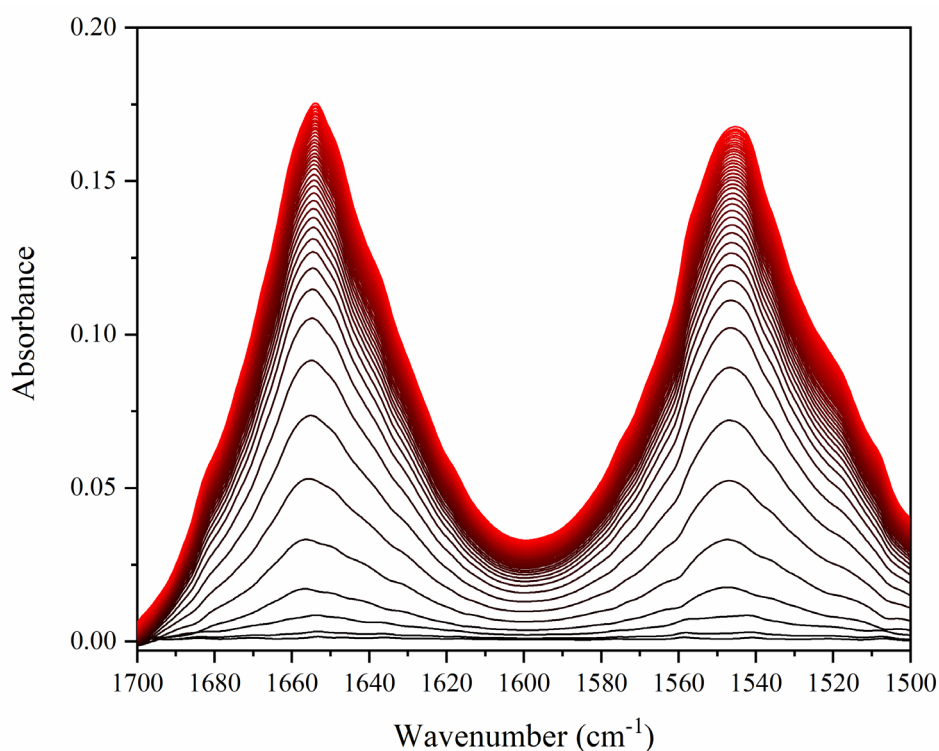


Figure2. 10: In situ ATR-FTIR measurements of BSA adsorption on TiO_2 thin film surface, 2 min between two successive spectra.

CHAPTER 2: BSA adsorption on porous TiO_2 Films - Kinetics study investigated by three states model -

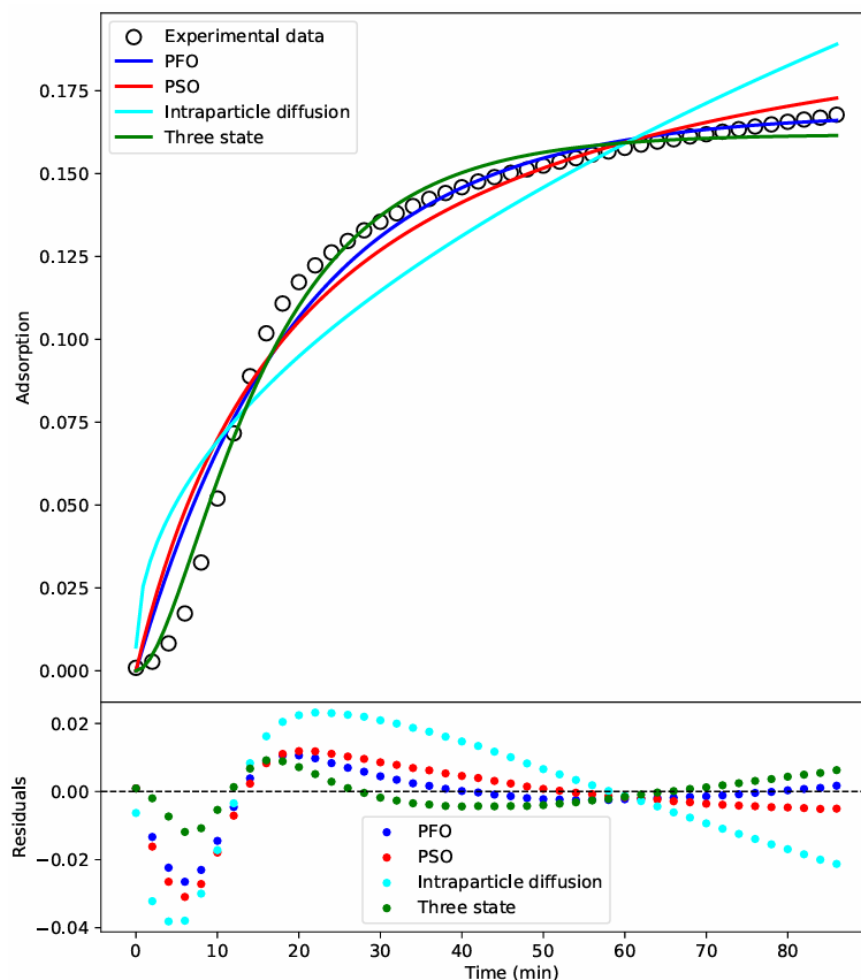


Figure2. 11: Fitting of Experimental Data Using Different Kinetic Models for BSA Adsorption on TiO_2 Surface.

Figure 2.11 represents fitting of the experimental kinetic data of BSA adsorption on titanium dioxide surface using different kinetic models (Pseudo-first-order model PFO, Pseudo-second-order model PSO, Intraparticle diffusion model, and Three-state model). It is observed that the experimental values increase rapidly in the early stages, meaning that the adsorption rate is high at the beginning due to the availability of a large surface area on the material, after which it gradually decreases until it reaches stability (surface saturation) reaching the value 0.170. This suggests that the studied adsorption cannot be explained by a simple reaction, but rather involves several physical states (a state in the solution, surface adsorption, and intraparticle diffusion). The three-state model achieves the best agreement with the experimental data compared to the other models, as confirmed by the lowest residual

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values. As for the residuals curve, it confirms this conclusion, as the three-state model gives residuals very close to zero, and this indicates high suitability, unlike the other models that show deviations, indicating the failure of the models to fully represent the kinetic system.

Table 2. 3: Kinetic parameters obtained from the fitting of BSA adsorption on titanium dioxide data using different kinetic models. The k value is k_1 (min⁻¹), k_2 (min⁻¹) and k_d (a.u. min^{-1/2}) in case of PFO, PSO and intraparticle diffusion respectively. All the parameters of the three state model have the same unite (min⁻¹).

Model	Fitted Parameters	R ²	χ^2	reduced χ^2
Pseudo-First-Order (PFO)	$k_1 = 0.0502$	0.9727	42.9300	1.0221
Pseudo-Second-Order (PSO)	$k_2 = 0.2260$	0.9578	42.6046	1.0144
Intraparticle Diffusion (IPD)	$k_d = 0.0196$; $C = 0.0072$	0.8697	42.0000	1.0000
Three-State Model	$K_{bas} = 0.000$; $K_{bd} = 0.1791$ $K_{dap} = 0.1003$; $K_{db} = 0.0341$	0.9904	40.2428	1.0061

The kinetic parameters and statistical fitting indicators derived from the four models are summarized in Table 2.3. Among the models evaluated, the three-state model demonstrated the best agreement with the experimental adsorption data, as evidenced by the highest coefficient of determination ($R^2 = 0.9904$) and the lowest χ^2 error value (40.2428), confirming that BSA adsorption onto TiO₂ proceeds through a complex, multi-stage mechanism rather than a single elementary process. The PFO model provided a reasonable fit to the experimental data ($R^2 = 0.9727$, $\chi^2 = 42.9300$), representing the second-best performance among the models tested. The PSO model yielded a comparably acceptable but slightly inferior fit ($R^2 = 0.9578$, $\chi^2 = 1.0144$). The intraparticle diffusion model exhibited the lowest predictive accuracy ($R^2 = 0.8697$), a result that nevertheless confirms that intraparticle diffusion contributes to, but does not solely govern, the overall adsorption kinetics. Taken together, these findings establish the three-state model as the most appropriate framework for describing the kinetics of BSA adsorption on TiO₂ surfaces.

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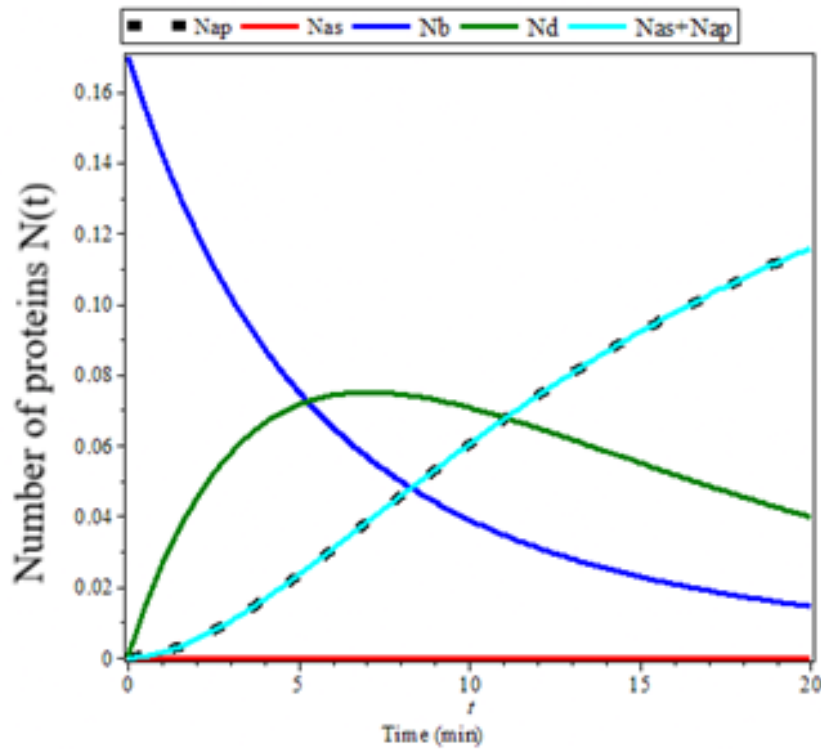


Figure2. 12: The curve represents the variation in the number of proteins in different states (bulk, surface adsorption, and pore adsorption) as a function of time.

The curve illustrates the temporal evolution of BSA molecules distributed among the bulk phase, the surface of titanium dioxide, and its internal pores. The number of proteins in the bulk phase (N_b) decreases rapidly with time as the molecules migrate from the solution toward the adsorbent material. In contrast, the number of proteins adsorbed within the pores (N_{ap}) increases progressively, reaching a maximum value of approximately 0.075 at $t = 6.5$, after which it gradually decreases. This behavior reflects the transfer and accumulation of proteins within the porous structure during the early stages of the adsorption process.

Meanwhile, the number of proteins adsorbed on the surface (N_{as}) remains close to zero throughout the experiment, indicating that surface adsorption is negligible compared to pore adsorption. Consequently, the total adsorbed amount ($N_{as} + N_{ap}$) is mainly governed by the evolution of N_{ap} . The predominance of pore adsorption suggests that the porous structure of titanium dioxide plays the major role in the adsorption process, with pore diffusion and retention being the dominant mechanisms controlling protein uptake.

7 Conclusion

This work investigated the kinetics of bovine serum albumin (BSA) adsorption on different surfaces, namely silicon, germanium, and titanium dioxide, where the main objective was to analyze the adsorption mechanism, understand the effect of surface nature on the kinetic behavior of the protein, and develop a more accurate kinetic model than the classical models capable of describing the multistage nature of the adsorption process.

A theoretical model for adsorption kinetics was developed and solved using MAPLE 17 software, and the numerical solution was integrated into a Python code. The proposed model was then compared with classical kinetic models (PFO, PSO, and the intraparticle diffusion model), and the results showed the limitations of these models in representing the full complexity of protein–surface interactions, while the three-state model showed excellent agreement with the experimental data, as demonstrated in the results and tables.

The study also showed that protein adsorption is a multistep process involving the transfer of molecules in the bulk solution, followed by surface adsorption, and then molecular rearrangement or diffusion depending on the nature of the surface. The results also highlighted the important role of surface nature in the adsorption mechanism, where flat surfaces such as silicon and germanium mainly rely on surface adsorption, whereas titanium dioxide introduces internal diffusion effects and higher adsorption capacity.

Overall, the proposed model provides an accurate and realistic description of protein adsorption and overcomes the limitations of classical models in explaining this complex phenomenon.

GENERAL CONCLUSION

General conclusion

In this work, we were interested in studying the adsorption of bovine serum albumin on different surfaces represented by silicon, germanium and titanium dioxide, with the aim of understanding the mechanism of protein interaction with different surfaces and the effect of porous structure on adsorption behavior. Where the theoretical study in the first chapter clarified the most important factors controlling the adsorption process such as surface nature and intermolecular interactions, as well as Fourier Transform Infrared Spectroscopy (FTIR) allowed to follow the adsorption process and to know the structural changes of proteins during interaction with surfaces. As for the second chapter it includes the applied side and it was used to analyze the adsorption kinetics using several kinetic models among them (pseudo-first order model, pseudo-second order model, Elovich model and intraparticle diffusion model) where the results showed the extent of limitation of these classical models considering that they are unable to represent the complex and multistep nature of protein adsorption on solid surfaces. This is what indicated the necessity to develop a model that provides an accurate and realistic description of the adsorption process by taking into account both surface adsorption and the transfer of molecules inside the porous structure where the results showed a better agreement of the proposed model with the experimental data compared to the other models. As well as the study showed the important role of surface nature in determining the adsorption mechanism as the porous titanium dioxide contributes to enhancing the adsorption capacity and showing effects related to the transport of molecules inside pores unlike non-porous surfaces (silicon and germanium) which mainly depend on surface adsorption.

At the end, this topic remains a broad and open field for further development, due to its great importance in biological and medical applications, and this confirms that continuing research in it is not a scientific choice but a necessity for a deeper understanding of biological interactions on surfaces and their realization in more effective applications

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Annexe I

The python code used to fit the experimental kinetics data

```
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
from matplotlib.gridspec import GridSpec
from scipy.optimize import curve_fit
from sklearn.metrics import r2_score
import os
os.chdir(r"C:\Users\j\Desktop\Master Project 2026\python code") # <-- PUT YOUR REAL PATH
HERE
print("Working directory set to:", os.getcwd())
def get_unique_filename(base_name, ext):
    i = 0
    filename = f"{base_name}{ext}"
    while os.path.exists(filename):
        i += 1
        filename = f"{base_name}_{i}{ext}"
    return filename
#Calling the data
sample = 'BSA_ad_Si'
ext = '.csv'
extpdf = '.pdf'
extcsv = '.csv'
filename = sample + ext
# Read the entire CSV file into a DataFrame
df = pd.read_csv(filename)
# Extract columns
time = df.iloc[:, 0].values # First column: Time
Max_absorbance = df.iloc[:, 1].values # Second column: Adsorption
#Pseudo-First-Order Fitting
# Define the adsorption model: Pseudo-First-Order
def pseudo_first_order(t, q_inf, k1):
    return q_inf * (1 - np.exp(-k1 * t))
# Initial parameter guesses (q_inf, k1)
initial_guess_PFO = [max(Max_absorbance), 0.01]
# Fit the model
params, covariance = curve_fit(pseudo_first_order, time, Max_absorbance, p0=initial_guess_PFO)
# Extract fitted parameters
q_inf_fit, k1_fit = params
print("===== PFO DATA =====")
print(f"PFO_Fitted parameters: q_inf = {q_inf_fit:.4f}, k1 = {k1_fit:.4f}")
# Generate fitted curve
time_fit = np.linspace(min(time), max(time), 100)
adsorption_fit_PFO = pseudo_first_order(time_fit, q_inf_fit, k1_fit)
# Calculate R-squared value
r2_PFO = r2_score(Max_absorbance, pseudo_first_order(time, q_inf_fit, k1_fit))
print(f"PFO_R-squared: {r2_PFO:.4f}")
# Compute residuals (difference between experimental and fitted values)
PFO_residuals = Max_absorbance - pseudo_first_order(time, q_inf_fit, k1_fit)
# Estimate variance (assuming uniform errors)
PFO_variance = np.var(PFO_residuals, ddof=2) # ddof=2 because we estimated 2 parameters (q_inf,
k)
# Compute chi-squared
PFO_chi_squared = np.sum((PFO_residuals**2) / PFO_variance)
```

```

# Degrees of freedom (DOF)
PFO_N = len(time) # Number of data points
PFO_p = 2 # Number of fitted parameters (q_inf, k1)
PFO_dof = PFO_N - PFO_p # Degrees of freedom
# Compute reduced chi-squared
PFO_chi_squared_reduced = PFO_chi_squared / PFO_dof
print(f"PFO_Chi-squared: {PFO_chi_squared:.4f}")
print(f"PFO_Reduced Chi-squared: {PFO_chi_squared_reduced:.4f}\n")
#Pseudo-Second-Order Fitting
# Define the adsorption model: Pseudo-Second-Order
def pseudo_second_order(t, q_inf, k2):
    return (q_inf**2 * k2 * t) / (1 + q_inf * k2 * t)
# Initial parameter guesses (q_inf, k2)
initial_guess_PSO = [max(Max_absorbance), 0.05]
# Fit the model
params, covariance = curve_fit(pseudo_second_order, time, Max_absorbance, p0=initial_guess_PSO)
# Extract fitted parameters
q_inf_fit, k2_fit = params
print("===== PSO DATA =====")
print(f"PSO_Fitted parameters: q_inf = {q_inf_fit:.4f}, k2 = {k2_fit:.4f}")
# Generate fitted curve
time_fit = np.linspace(min(time), max(time), 100)
adsorption_fit_PSO = pseudo_second_order(time_fit, q_inf_fit, k2_fit)
# Calculate R-squared value
r2_PSO = r2_score(Max_absorbance, pseudo_second_order(time, q_inf_fit, k2_fit))
print(f"PSO_R-squared: {r2_PSO:.4f}")
# Compute residuals (difference between experimental and fitted values)
PSO_residuals = Max_absorbance - pseudo_second_order(time, q_inf_fit, k2_fit)
# Estimate variance (assuming uniform errors)
PSO_variance = np.var(PSO_residuals, ddof=2) # ddof=2 because we estimated 2 parameters (q_inf, k)
# Compute chi-squared
PSO_chi_squared = np.sum((PSO_residuals**2) / PSO_variance)
# Degrees of freedom (DOF)
PSO_N = len(time) # Number of data points
PSO_p = 2 # Number of fitted parameters (q_inf, k)
PSO_dof = PSO_N - PSO_p # Degrees of freedom
# Compute reduced chi-squared
PSO_chi_squared_reduced = PSO_chi_squared / PSO_dof
print(f"PSO_Chi-squared: {PSO_chi_squared:.4f}")
print(f"PSO_Reduced Chi-squared: {PSO_chi_squared_reduced:.4f}\n")
#intraparticle diffusion Fitting
# Define the adsorption model: Intraparticle Diffusion (Weber-Morris)
def intraparticle_diffusion(t, kid, C):
    return kid * np.sqrt(t) + C
# Initial parameter guesses (kid, C)
initial_guess_IPD = [max(Max_absorbance) / np.sqrt(max(time)), 0.0]
# Fit the model
params_IPD, covariance_IPD = curve_fit(intraparticle_diffusion, time, Max_absorbance, p0=initial_guess_IPD)
# Extract fitted parameters
kid_fit, C_fit = params_IPD
print("===== Intraparticle Diffusion DATA =====")
print(f"IPD_Fitted parameters: kid = {kid_fit:.4f}, C = {C_fit:.4f}")
# Generate fitted curve
time_fit_IPD = np.linspace(min(time), max(time), 100)

```

```

adsorption_fit_IPD = intraparticle_diffusion(time_fit_IPD, kid_fit, C_fit)
# Calculate R-squared value
r2_IPD = r2_score(Max_ absorbance, intraparticle_diffusion(time, kid_fit, C_fit))
print(f'IPD_R-squared: {r2_IPD:.4f}')
# Compute residuals
IPD_residuals = Max_ absorbance - intraparticle_diffusion(time, kid_fit, C_fit)
# Estimate variance
IPD_variance = np.var(IPD_residuals, ddof=2) # ddof=2 for 2 parameters (kid, C)
# Compute chi-squared
IPD_chi_squared = np.sum((IPD_residuals**2) / IPD_variance)
# Degrees of freedom
IPD_N = len(time)
IPD_p = 2 # Number of fitted parameters (kid, C)
IPD_dof = IPD_N - IPD_p
# Compute reduced chi-squared
IPD_chi_squared_reduced = IPD_chi_squared / IPD_dof
print(f'IPD_Chi-squared: {IPD_chi_squared:.4f}')
print(f'IPD_Reduced Chi-squared: {IPD_chi_squared_reduced:.4f}\n')
#three state model fitting
def three_state_scaled(t, A, Kbas, Kbd, Kdap, Kdb):
    # --- Core reusable term ---
    Q = (
        Kbas**2 + 2*Kbas*Kbd - 2*Kbas*Kdap - 2*Kbas*Kdb
        + Kbd**2 - 2*Kbd*Kdap + 2*Kbd*Kdb
        + Kdap**2 + 2*Kdap*Kdb
        + Kdb**2
    )
    Q = np.maximum(Q, 1e-12)
    sqrtQ = np.sqrt(Q)
    # --- Second sqrt term ---
    inner = (
        Kbas**2
        + (-2*Kdap - 2*Kdb + 2*Kbd)*Kbas
        + Kdap**2
        + (-2*Kbd + 2*Kdb)*Kdap
        + (Kbd + Kdb)**2
    )
    inner = np.maximum(inner, 1e-12)
    sqrt_inner = np.sqrt(inner)
    # --- Exponential rates ---
    lam1 = 0.5 * (Kbas + Kbd + Kdap + Kdb - sqrt_inner)
    lam2 = 0.5 * (Kbas + Kbd + Kdap + Kdb + sqrt_inner)
    # --- Common denominator ---
    denom1 = Q * (-0.5*(Kbas + Kbd + Kdap + Kdb) + 0.5*sqrt_inner)
    denom2 = Q * (-0.5*(Kbas + Kbd + Kdap + Kdb) - 0.5*sqrt_inner)
    denom1 = np.where(np.abs(denom1) < 1e-12, 1e-12, denom1)
    denom2 = np.where(np.abs(denom2) < 1e-12, 1e-12, denom2)
    # --- Term 1 ---
    term1 = (
        0.5 * Kdap *
        ((2/25)*(Q - Kbas*sqrtQ - Kbd*sqrtQ + Kdap*sqrtQ + Kdb*sqrtQ)
        * (Kbd - Kdb + Kbas - Kdap + sqrt_inner)
        * np.exp(-lam1 * t) / denom1)
    )
    # --- Term 2 ---
    term2 = (

```

```

0.5 * Kdap *
((2/25)*(Q + Kbas*sqrtQ + Kbd*sqrtQ - Kdap*sqrtQ - Kdb*sqrtQ)
 * (Kbd - Kdb + Kbas - Kdap - sqrt_inner)
 * np.exp(-lam2 * t) / denom2)
)
# --- Static term ---
term3 = (4/25) * Kbd * Kdap / (Kbas*Kdap + Kbas*Kdb + Kbd*Kdap + 1e-12)
# --- Kbas-dependent term ---
term4 = (
    Kbas *
    ((2/25)*(Q - Kbas*sqrtQ - Kbd*sqrtQ + Kdap*sqrtQ + Kdb*sqrtQ)
     * np.exp(-lam1 * t) / denom1
    + (2/25)*(Q + Kbas*sqrtQ + Kbd*sqrtQ - Kdap*sqrtQ - Kdb*sqrtQ)
     * np.exp(-lam2 * t) / denom2)
)

# --- Final constant ---
term5 = (4/25) * Kbas * (Kdap + Kdb) / (Kbas*Kdap + Kbas*Kdb + Kbd*Kdap + 1e-12)
# --- FINAL (scaled) ---
return A * ((term1 + term2) / (Kdb + 1e-12) + term3 + term4 + term5)
# =====
initial_guess_three_state = [max(Max_ absorbance), 0.1, 0.1, 0.1, 0.1]
params, covariance = curve_fit(
    three_state_scaled,
    time,
    Max_ absorbance,
    p0=initial_guess_three_state,
    bounds=([0, 0, 0, 0, 0], [np.inf, np.inf, np.inf, np.inf, np.inf]), # rates should be positive
    maxfev=50000
)
A_fit, Kbas_fit, Kbd_fit, Kdap_fit, Kdb_fit = params
print("===== Three state DATA =====")
print(f'Three_state_Fitted parameters: Kbas={Kbas_fit:.4f}, Kbd={Kbd_fit:.4f},
Kdap={Kdap_fit:.4f}, Kdb={Kdb_fit:.4f}')

adsorption_fit_model1 = three_state_scaled(time_fit, A_fit, Kbas_fit, Kbd_fit, Kdap_fit, Kdb_fit)
# R2
r2_three_state = r2_score(Max_ absorbance, three_state_scaled(time, *params))
print(f'three_state_R-squared: {r2_three_state:.4f}')
# Residuals
three_state_residuals = Max_ absorbance - three_state_scaled(time, *params)
three_state_variance = np.var(three_state_residuals, ddof=4)
three_state_chi_squared = np.sum((three_state_residuals**2) / three_state_variance)
three_state_dof = len(time) - 4
print(f'three_state_Chi-squared: {three_state_chi_squared:.4f}')
print(f'three_state_Reduced Chi-squared: {three_state_chi_squared/three_state_dof:.4f}\n')
#fig, (ax_plot, ax_res, ax_text) = plt.subplots(
    # 3, 1, figsize=(8, 12),
    # sharex=True,
    # gridspec_kw={'height_ratios': [3, 1, 1], 'hspace': 0.01}
#)
fig = plt.figure(figsize=(8, 12))
gs = GridSpec(3, 1, height_ratios=[3, 1, 1], hspace=0)

ax_plot = fig.add_subplot(gs[0])
ax_res = fig.add_subplot(gs[1], sharex=ax_plot)

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ax_text = fig.add_subplot(gs[2])
# Now manually push ax_text down to create gap only between residuals and text
pos = ax_text.get_position()
ax_text.set_position([pos.x0, pos.y0 - 0.05, pos.width, pos.height]) # adjust 0.05
# =====
# TOP: MAIN PLOT
# =====
ax_plot.scatter(
    time,
    Max_absorbance,
    facecolors='none',
    edgecolors='black',
    linewidths=1.2,
    s=70,
    label="Experimental data"
)
ax_plot.plot(time_fit, adsorption_fit_PFO, color='blue',linestyle=(0, (5,8)),alpha=0.5, linewidth=2,
label="PFO")
ax_plot.plot(time_fit, adsorption_fit_PSO, color='red', linewidth=2, label="PSO")
ax_plot.plot(time_fit, adsorption_fit_IPD, color='cyan', linewidth=2, label="Intraparticle diffusion")
ax_plot.plot(time_fit, adsorption_fit_model1, color='green',linestyle=(0, (5,15)), linewidth=2,
label="Three state")

ax_plot.set_ylabel("Adsorption")
ax_plot.legend()
# Add tick labels only on the residuals plot
ax_res.tick_params(labelbottom=True) # make sure ticks are visible
# Set the tick positions and labels
ax_res.set_xticks(np.arange(0, max(time)+1, 2)) # adjust step (2) to your data range
# =====
# MIDDLE: RESIDUALS (ALL MODELS)
# =====
ax_res.scatter(time, PFO_residuals, color='blue', s=15, label="PFO")
ax_res.scatter(time, PSO_residuals, color='red', s=15, label="PSO")
ax_res.scatter(time, IPD_residuals, color='cyan', s=15, label="Intraparticle diffusion")
ax_res.scatter(time, three_state_residuals, color='green', s=15, label="Three state")
# Zero reference line
ax_res.axhline(0, color='black', linestyle='--', linewidth=1)
ax_res.set_ylabel("Residuals")
ax_res.set_xlabel("Time (min)")
ax_res.legend()
# =====
# BOTTOM: TEXT RESULTS
# =====
textstr = (
    "===== PFO DATA =====\n"
    f"PFO_Fitted parameters: q_inf = {q_inf_fit:.4f}, k1 = {k1_fit:.4f}\n"
    f"PFO_R-squared: {r2_PFO:.4f}\n"
    f"PFO_Chi-squared: {PFO_chi_squared:.4f}\n"
    f"PFO_Reduced Chi-squared: {PFO_chi_squared_reduced:.4f}\n\n"
    "===== PSO DATA =====\n"
    f"PSO_Fitted parameters: q_inf = {q_inf_fit:.4f}, k2 = {k2_fit:.4f}\n"
    f"PSO_R-squared: {r2_PSO:.4f}\n"
    f"PSO_Chi-squared: {PSO_chi_squared:.4f}\n"
    f"PSO_Reduced Chi-squared: {PSO_chi_squared_reduced:.4f}\n\n"
    "===== IPD DATA =====\n"

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f"IPD_Fitted parameters: kid = {kid_fit:.4f}, C = {C_fit:.4f}\n"
f"IPD_R-squared: {r2_IPD:.4f}\n"
f"IPD_Chi-squared: {IPD_chi_squared:.4f}\n"
f"IPD_Reduced Chi-squared: {IPD_chi_squared_reduced:.4f}\n\n"
"===== Three state DATA =====\n"
f"Three_state_Fitted parameters: Kbas={Kbas_fit:.4f}, Kbd={Kbd_fit:.4f}, "
f"Kdap={Kdap_fit:.4f}, Kdb={Kdb_fit:.4f}\n"
f"three_state_R-squared: {r2_three_state:.4f}\n"
f"three_state_Chi-squared: {three_state_chi_squared:.4f}\n"
f"three_state_Reduced Chi-squared: {three_state_chi_squared/three_state_dof:.4f}\n"
)
ax_text.axis('off')
ax_text.text(
    0.01, 0.95,
    textstr,
    fontsize=9,
    family='monospace',
    verticalalignment='top'
)
# =====
# SAVE FIGURE
# =====
pdf_filename = get_unique_filename(sample, ".pdf")
fig.savefig(pdf_filename, bbox_inches='tight')
print("Saved as:", pdf_filename)
# =====
# SHOW FIGURE
# =====
plt.show()

```